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~~Title of Invention~~: → BRETT B. FINLAY; BRENDANT KENNY;
Inventors (please provide full names): REBEKAH DE VINNEY; MARCUS STEIN.

Earliest Priority Date: 11.12.97

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Please perform an inventor's name search.

Point of Contact:
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Technical Info. Specialist
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_____ Other (specify)

Devi, S.
09/189415

- key terms

FILE 'REGISTRY' ENTERED AT 11:47:39 ON 28 SEP 2001
E TRANSLOCATED INTIMIN RECEPTOR/CN
L3 3 SEA ABB=ON PLU=ON TRANSLOCATED INTIMIN RECEPTOR ?/CN

L11 18 S INTIMIN ?/CN

FILE 'CAPLUS' ENTERED AT 14:19:36 ON 28 SEP 2001
L1 (2348) SEA FILE=CAPLUS ABB=ON PLU=ON 90KD? OR 90(W)(KD? OR KILOD? OR KILO(W)(D OR DA OR DALTON))
L2 (1011) SEA FILE=CAPLUS ABB=ON PLU=ON L1(5A)PROTEIN
L3 (3) SEA FILE=REGISTRY ABB=ON PLU=ON TRANSLOCATED INTIMIN RECEPTOR ?/CN
L4 1609 SEA FILE=CAPLUS ABB=ON PLU=ON L2 OR L3 OR (TRANSLOCAT? OR TRANS LOCAT?)(W)INTIMIN(W)RECEPTOR OR TIR OR HP90 OR HP 90
L5 1209 SEA FILE=CAPLUS ABB=ON PLU=ON L4 AND (PROTEIN OR POLYPEPTIDE OR POLYPROTEIN OR PEPTIDE)
L6 70 SEA FILE=CAPLUS ABB=ON PLU=ON L5 AND (EPEC OR EHEC OR "A/E" OR ATTACH?(1W)EFFAC? OR (ENTEROPATHOGEN? OR ENTEROHEMORRH? OR ENTEROHAEMORRH? OR ENTERO(W)(PATHOGEN? OR HEMORRH? OR HAEMORRH?))(5A)COLI)
L11 18 SEA FILE=REGISTRY ABB=ON PLU=ON INTIMIN ?/CN
L12 57 SEA FILE=CAPLUS ABB=ON PLU=ON L6 AND (L11 OR INTIMIN)
L13 34 SEA FILE=CAPLUS ABB=ON PLU=ON L12 AND BIND?

L13 ANSWER 1 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:699619 CAPLUS
TITLE: **Enteropathogenic** E. **coli** **tir binds** Nck to initiate actin pedestal formation in host cells
AUTHOR(S): Gruenheid, Samantha; DeVinney, Rebekah; Bladt, Friedhelm; Goosney, Danika; Gelkop, Sigal; Gish, Gerald D.; Pawson, Tony; Finlay, B. Brett
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1G3, Can.
SOURCE: Nat. Cell Biol. (2001), 3(9), 856-859
CODEN: NCBIFN; ISSN: 1465-7392
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Enteropathogenic** Escherichia **coli** (**EPEC**) is a bacterial pathogen that causes infantile diarrhea worldwide. **EPEC** injects a bacterial **protein**, **translocated intimin receptor** (**Tir**), into the host-cell plasma membrane where it acts as a receptor for the bacterial outer membrane **protein**, **intimin**. The interaction of **Tir** and **intimin** triggers a marked rearrangement of the host actin

cytoskeleton into pedestals beneath adherent bacteria. On delivery into host cells, **EPEC Tir** is phosphorylated on tyrosine 474 of the intracellular carboxy-terminal domain, an event that is required for pedestal formation. Despite its essential role, the function of **Tir** tyrosine phosphorylation has not yet been elucidated. Here we show that tyrosine 474 of **Tir** directly **binds** the host-cell adaptor **protein** Nck, and that Nck is required for the recruitment of both neural Wiskott-Aldrich-syndrome **protein** (N-WASP) and the actin-related **protein** (Arp)2/3 complex to the **EPEC** pedestal, directly linking **Tir** to the cytoskeleton. Cells with null alleles of both mammalian Nck genes are resistant to the effects of **EPEC** on the actin cytoskeleton. These results implicate Nck adaptors as host-cell determinants of **EPEC** virulence.

L13 ANSWER 2 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:642816 CAPLUS
TITLE: **Intimin**-specific immune responses prevent bacterial colonization by the **attaching-effacing** pathogen Citrobacter rodentium
AUTHOR(S): Ghaem-Maghami, Marjan; Simmons, Cameron P.; Daniell, Sarah; Pizza, Mariagrazia; Lewis, David; Frankel, Gad; Dougan, Gordon
CORPORATE SOURCE: Centre for Molecular Microbiology and Infection, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7 2AZ, UK
SOURCE: Infect. Immun. (2001), 69(9), 5597-5605
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The formation of **attaching** and **effacing** (**A/E**) lesions on gut enterocytes is central to the pathogenesis of **enterohemorrhagic** (**EHEC**) Escherichia **coli**, **enteropathogenic** E. **coli** (**EPEC**), and the rodent pathogen Citrobacter rodentium. Genes encoding **A/E** lesion formation map to a chromosomal pathogenicity island termed the locus of enterocyte effacement (LEE). Here we show that the LEE-encoded **proteins** EspA, EspB, **Tir**, and **intimin** are the targets of long-lived humoral immune responses in C. rodentium-infected mice. Mice infected with C. rodentium developed robust acquired immunity and were resistant to reinfection with wild-type C. rodentium or a C. rodentium deriv., DBS255(pCVD438), which expressed **intimin** derived from **EPEC** strain

E2348/69. The receptor-**binding** domain of **intimin** **polypeptides** is located within the carboxy-terminal 280 amino acids (Int280). Mucosal and systemic vaccination regimens using enterotoxin-based adjuvants were employed to elicit immune responses to recombinant Int280.alpha. from **EPEC** strain E2348/69. Mice vaccinated s.c. with Int280.alpha., in the absence of adjuvant, were significantly more resistant to oral challenge with DBS255(pCVD438) but not with wild-type C. rodentium. This type-specific immunity could not be overcome by employing an exposed, highly conserved domain of **intimin** (Int388-667) as a vaccine. These results show that anti-**intimin** immune responses can modulate the outcome of a C. rodentium infection and support the use of **intimin** as a component of a type-specific **EPEC** or **EHEC** vaccine.

REFERENCE COUNT: 45

REFERENCE(S):
(1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
(2) An, H; Microb Pathog 2000, V28, P291 CAPLUS
(5) Batchelor, M; J Clin Microbiol 1999, V37, P3822 CAPLUS
(7) Bouvet, J; Infect Immun 1999, V67, P2687 CAPLUS
(8) China, B; Res Microbiol 1999, V150, P323 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 3 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2001:341382 CAPLUS

DOCUMENT NUMBER: 135:42490

TITLE: **Intimin** from Shiga toxin-producing Escherichia coli and its isolated C-terminal domain exhibit different **binding** properties for **Tir** and a eukaryotic surface receptor

AUTHOR(S): Deibel, Christina; Dersch, Petra; Ebel, Frank

CORPORATE SOURCE: Institut fur Medizinische Mikrobiologie, Justus-Liebig-Universitat, Giessen, Germany

SOURCE: Int. J. Med. Microbiol. (2001), 290(8), 683-691
CODEN: IMEMFV; ISSN: 1438-4221

PUBLISHER: Urban & Fischer Verlag

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The outer membrane **protein** **intimin** plays a crucial role in the **attaching** and **effacing** process employed by different enteropathogens to colonize the epithelial surface of their hosts. In this study the authors have characterized the C-terminal **binding** domain of **intimin** from the Shiga toxin-producing E. coli strain

413/89-1, that belongs to the .beta.-subtype of **intimins**. The authors found that a fusion of this domain to the maltose-**binding protein binds** efficiently to both the **translocated intimin receptor** (**Tir**) and the surface of uninfected eukaryotic host cells. In contrast, no such **binding** was obsd. with the full-length **protein** localized on the bacterial surface. As the C-terminal domain of **intimin** and the full-length **protein** differ in their **binding** activity, the authors suggest that the **intimin-binding** domain might be controlled by the N-terminal portion of the mol. to prevent unproductive interactions with mols. in the lumen of the gut.

REFERENCE COUNT: 30

REFERENCE(S):
(1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
(2) Celli, J; Cell Microbiol 2000, V2, P1 CAPLUS
(3) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS
(4) Dersch, P; EMBO J 1999, V18, P1199 CAPLUS
(5) Devinney, R; Cell Mol Life Sci 1999, V55, P961 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 4 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2001:309935 CAPLUS

DOCUMENT NUMBER: 135:91417

TITLE: Recruitment of cytoskeletal and signaling **proteins** to **enteropathogenic** and **enterohemorrhagic** Escherichia **coli** pedestals

AUTHOR(S): Goosney, Danika L.; DeVinney, Rebekah; Finlay, B. Brett

CORPORATE SOURCE: Biotechnology Laboratory and Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Infect. Immun. (2001), 69(5), 3315-3322
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) is a human pathogen that attaches to intestinal epithelial cells and causes chronic watery diarrhea. A close relative, **enterohemorrhagic** E. **coli** (**EHEC**), causes severe bloody diarrhea and hemolytic-uremic syndrome. Both pathogens insert a **protein**, **Tir**, into the host cell plasma membrane where it **binds intimin**, the outer membrane ligand of **EPEC** and **EHEC**. This

interaction triggers a cascade of signaling events within the host cell and ultimately leads to the formation of an actin-rich pedestal upon which the pathogen resides. Pedestal formation is crit. in mediating **EPEC**- and **EHEC**-induced diarrhea, yet very little is known about its compn. and organization. In **EPEC**, pedestal formation requires **Tir** tyrosine 474 phosphorylation. In **EHEC Tir** is not tyrosine phosphorylated, yet the pedestals appear similar. The compn. of the **EPEC** and **EHEC** pedestals was analyzed by examg. numerous cytoskeletal, signaling, and adapter **proteins**. Of the 25 **proteins** examd., only two, calpactin and CD44, were recruited to the site of bacterial attachment independently of **Tir**. Several others, including ezrin, talin, gelsolin, and tropomyosin, were recruited to the site of **EPEC** attachment independently of **Tir** tyrosine 474 phosphorylation but required **Tir** in the host membrane. The remaining **proteins** were recruited to the pedestal in a manner dependent on **Tir** tyrosine phosphorylation or were not recruited at all. Differences were also found between the **EPEC** and **EHEC** pedestals: the adapter **proteins** Grb2 and CrkII were recruited to the **EPEC** pedestal but were absent in the **EHEC** pedestal. These results demonstrate that although **EPEC** and **EHEC** recruit similar cytoskeletal **proteins**, there are also significant differences in pedestal compn.

REFERENCE COUNT: 44

REFERENCE(S):
(1) Adam, T; J Cell Biol 1995, V129, P367 CAPLUS
(2) Ben-Ami, G; Infect Immun 1998, V66, P1755 CAPLUS
(4) Burridge, K; Nature 1984, V308, P744 CAPLUS
(5) Cantarelli, V; Infect Immun 2000, V68, P382 CAPLUS
(6) Carlier, M; J Biol Chem 2000, V275, P21946 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 5 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2001:296138 CAPLUS

DOCUMENT NUMBER: 135:90741

TITLE: Site-directed mutagenesis of **intimin** .alpha. modulates **intimin**-mediated tissue tropism and host specificity

AUTHOR(S): Reece, Stephen; Simmons, Cameron P.; Fitzhenry, Robert J.; Matthews, Stephen; Phillips, Alan D.; Dougan, Gordon; Frankel, Gad

CORPORATE SOURCE: Centre for Molecular Microbiology and Infection, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7

2AZ, UK
SOURCE: Mol. Microbiol. (2001), 40(1), 86-98
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The hallmark of **enteropathogenic** (**EPEC**) and **enterohemorrhagic** (**EHEC**) Escherichia **coli** adhesion to host cells is intimate attachment leading to the formation of distinctive "**attaching** and **effacing** " lesions. This event is mediated, in part, by **binding** of the bacterial adhesion mol. **intimin** to a second bacterial **protein**, **Tir**, delivered by a type III secretion system into the host cell plasma membrane. The receptor-**binding** activity of **intimin** is localized to the C-terminal 280 amino acids (Int280) and at least five distinct **intimin** types (.alpha., .beta., .gamma., .delta. and .epsilon.) have been identified thus far. In addn. to **binding** to **Tir**, **intimin** can also **bind** to a component encoded by the host. The consequence of latter **intimin-binding** activity may det. tissue tropism and host specificity. In this study the authors selected three amino acids in **intimin**, which are implicated in **Tir binding**, for site-directed mutagenesis. The authors used the yeast two-hybrid system and gel overlays to study **intimin-Tir protein** interaction. In addn., the biol. consequences of the mutagenesis was tested using a no. of infection models (cultured epithelial cells, human intestinal explants and a mouse model). The authors report that while an I237/897A substitution (positions numbered according to Int280.alpha./whole **intimin** .alpha.) in **intimin** .alpha. did not have any affect on its biol. activity, a T255/914A substitution attenuated **intimin** activity in vivo. In contrast, the mutation V252/911A affected tissue targeting in the human intestinal explant model and attenuated the biol. activity of **intimin** in the mouse model. This study provides the first clues of the mol. basis of how **intimin** mediates tissue tropism and host specificity.

REFERENCE COUNT: 62
REFERENCE(S):
(1) Abe, A; Mol Microbiol 1999, V33, P1162 CAPLUS
(2) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
(3) An, H; FEMS Microbiol Lett 1997, V148, P239 CAPLUS
(5) Batchelor, M; EMBO J 2000, V19, P2452 CAPLUS
(6) de Grado, M; Cell Microbiol 1999, V1, P7 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 6 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:184271 CAPLUS
DOCUMENT NUMBER: 134:217892
TITLE: Complete genome sequence of enterohemorrhagic Escherichia coli O157:H7 and genomic comparison with a laboratory strain K-12
AUTHOR(S): Hayashi, Tetsuya; Makino, Kozo; Ohnishi, Makoto; Kurokawa, Ken; Ishii, Kazuo; Yokoyama, Katsushi; Han, Chang-Gyun; Ohtsubo, Eiichi; Nakayama, Keisuke; Murata, Takahiro; Tanaka, Masashi; Tobe, Toru; Iida, Tetsuya; Takami, Hideto; Honda, Takeshi; Sasakawa, Chihiro; Ogasawara, Naotake; Yasunaga, Teruo; Kuhara, Satoru; Shiba, Tadayoshi; Hattori, Masahira; Shinagawa, Hideo
CORPORATE SOURCE: Department of Microbiology, Miyazaki Medical College, Miyazaki, 899-1692, Japan
SOURCE: DNA Res. (2001), 8(1), 11-22
CODEN: DARSE8; ISSN: 1340-2838
PUBLISHER: Universal Academy Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Escherichia coli O157:H7 is a major food-borne infectious pathogen that causes diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. The complete chromosome sequence of an O157:H7 strain isolated from the Sakai outbreak is reported, and the results compared with the genome of a benign lab. strain, K-12 MG1655. The chromosome is 5.5 Mb in size, 859 Kb larger than that of K-12. A 4.1-Mb sequence highly conserved between the two strains is identified, which may represent the fundamental backbone of the E. coli chromosome. The remaining 1.4-Mb sequence comprises of O157:H7-specific sequences, most of which are horizontally transferred foreign DNAs. The predominant roles of bacteriophages in the emergence of O157:H7 is evident by the presence of 24 prophages and prophage-like elements that occupy more than half of the O157:H7-specific sequences. The O157:H7 chromosome encodes 1632 proteins and 20 tRNAs that are not present in K-12. Among these, at least 131 proteins are assumed to have virulence-related functions. Genome-wide codon usage anal. suggested that the O157:H7-specific tRNAs are involved in the efficient expression of the strain-specific genes. A complete set of the genes specific to O157:H7 presented here sheds new insight into the pathogenicity and the physiol. of O157:H7, and will open a way to fully understand the mol. mechanisms underlying the O157:H7 infection.

REFERENCE COUNT: 62
REFERENCE(S): (1) Altschul, S; Nucleic Acids Res 1997, V25, P3389 CAPLUS

(2) Amor, K; Infect Immun 2000, V68, P1116
CAPLUS
(5) Bergthorsson, U; Mol Biol Evol 1998, V15, P6
CAPLUS
(6) Blattner, F; Science 1997, V277, P1453
CAPLUS
(9) Casjens, S; Mol Microbiol 2000, V35, P490
CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 7 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:98372 CAPLUS
DOCUMENT NUMBER: 134:232542
TITLE: Genome sequence of enterohemorrhagic Escherichia coli O157:H7
AUTHOR(S): Perna, Nicole T.; Plunkett, Guy, III; Burland, Valerie; Mau, Bob; Glasner, Jeremy D.; Rose, Debra J.; Mayhew, George F.; Evans, Peter S.; Gregor, Jason; Kirkpatrick, Heather A.; Posfai, Gyorgy; Hackett, Jeremiah; Klink, Sara; Boutin, Adam; Shao, Ying; Miller, Leslie; Grotbeck, Erik J.; Davis, N. Wayne; Lim, Alex; Dimalanta, Eileen T.; Potamousis, Konstantinos D.; Apodaca, Jennifer; Anantharaman, Thomas S.; Lin, Jieyi; Yen, Glaex; Schwartz, David C.; Welch, Rodney A.; Blattner, Frederick R.
CORPORATE SOURCE: Genome Center of Wisconsin, Department of Animal Health and Biomedical Sciences, Laboratory of Genetics, Department of Chemistry, Department of Biostatistics, and Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, WI, 53706, USA
SOURCE: Nature (London) (2001), 409(6819), 529-533
CODEN: NATUAS; ISSN: 0028-0836
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The bacterium Escherichia coli O157:H7 is a worldwide threat to public health and has been implicated in many outbreaks of hemorrhagic colitis, some of which included fatalities caused by hemolytic uremic syndrome. Close to 75,000 cases of O157:H7 infection are now estd. to occur annually in the United States. The severity of disease, the lack of effective treatment and the potential for large-scale outbreaks from contaminated food supplies have propelled intensive research on the pathogenesis and detection of E. coli O157:H7. The genome of E. coli O157:H7 was sequenced to identify candidate genes responsible for pathogenesis, to develop better methods of strain detection and to advance our understanding

of the evolution of E. coli, through comparison with the genome of the non-pathogenic lab. strain E. coli K-12. Lateral gene transfer found to be far more extensive than previously anticipated. In fact, 1387 new genes encoded in strain-specific clusters of diverse sizes were found in O157:H7. These include candidate virulence factors, alternative metabolic capacities, several prophages, and other new functions - all of which could be targets for surveillance.

REFERENCE COUNT: 30

REFERENCE(S):
(1) Alm, R; J Mol Med 1999, V77, P834 CAPLUS
(2) Altschul, S; J Mol Biol 1990, V215, P403 CAPLUS
(3) Blaisdell, J; J Bacteriol 1999, V181, P6396 CAPLUS
(5) Blattner, F; Science 1997, V277, P1453 CAPLUS
(6) Boyd, E; J Bacteriol 1997, V179, P1985 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 8 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:899327 CAPLUS

DOCUMENT NUMBER: 134:204805

TITLE: Interaction of the **enteropathogenic** Escherichia **coli protein, translocated intimin receptor** (**tir**), with focal adhesion **proteins**

AUTHOR(S): Freeman, Nancy L.; Zurawski, Daniel V.; Chowrashi, Prokash; Ayoob, Joseph C.; Huang, Lily; Mittal, Balraj; Sanger, Jean M.; Sanger, Joseph W.

CORPORATE SOURCE: Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104-6058, USA

SOURCE: Cell Motil. Cytoskeleton (2000), 47(4), 307-318
CODEN: CMCYEO; ISSN: 0886-1544

PUBLISHER: Wiley-Liss, Inc.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB When **enteropathogenic** Escherichia **coli** (**EPEC**) attach and infect host cells, they induce a cytoskeletal rearrangement and the formation of cytoplasmic columns of actin filaments called pedestals. The attached **EPEC** and pedestals move over the surface of the host cell in an actin-dependent reaction [Sanger et al., 1996: Cell Motil Cytoskeleton 34:279-287]. The discovery that **EPEC** inserts the **protein, translocated intimin**

receptor (**Tir**), into the membrane of host cells, where it **binds** the **EPEC** outer membrane **protein**, **intimin** [Kenny et al., 1997: Cell 91:511-520], suggests **Tir** serves two functions: tethering the bacteria to the host cell and providing a direct connection to the host's cytoskeleton. The sequence of **Tir** predicts a **protein** of 56.8 kD with three domains sepd. by two predicted trans-membrane spanning regions. A GST-fusion **protein** of the N-terminal 233 amino acids of **Tir** (Tir1) **binds** to alpha-actinin, talin, and vinculin from cell exts. GST-Tir1 also coppts. purified forms of alpha-actinin, talin, and vinculin while GST alone does not **bind** these three focal adhesion **proteins**. Biotinylated probes of these three **proteins** also bound Tir1 cleaved from GST. Similar assocns. of alpha-actinin, talin, and vinculin were also detected with the C-terminus of **Tir**, i.e., Tir3, the last 217 amino acids. Antibody staining of **EPEC**-infected cultured cells reveals the presence of focal adhesion **proteins** beneath the attached bacteria. Our expts. support a model in which the cytoplasmic domains of **Tir** recruit a no. of focal adhesion **proteins** that can **bind** actin filaments to form pedestals. Since pedestals also contain villin, tropomyosin and myosin II [Sanger et al., 1996: Cell Motil. Cytoskeleton 34:279-287], the pedestals appear to be a novel structure sharing properties of both focal adhesions and microvilli.

REFERENCE COUNT: 31

REFERENCE(S):

(1) Ayoob, J; Cell Motil Cytoskeleton 2000, V45, P67 CAPLUS
(2) Bourdet-Sicard, R; EMBO J 1999, V18, P5853 CAPLUS
(3) Chen, B; Mol Biol Cell 1999, V10, P4327 CAPLUS
(4) Critchley, D; Biochem Soc Symp 1999, V65, P79 CAPLUS
(5) Dabiri, G; Proc Natl Acad Sci USA 1990, V87, P6068 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 9 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:603715 CAPLUS

DOCUMENT NUMBER: 133:280269

TITLE: Human response to Escherichia coli O157:H7 infection: antibodies to secreted virulence factors

AUTHOR(S): Li, Yuling; Frey, Elizabeth; Mackenzie, Andrew M. R.; Finlay, B. Brett

CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Infect. Immun. (2000), 68(9), 5090-5095
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Vaccination has been proposed for the prevention of disease due to **enterohemorrhagic** Escherichia **coli** (**EHEC**), but the immune response following human infection, including the choice of potential antigens, has not been well characterized. To study this, sera were obtained from five pediatric patients with acute diarrhea caused by E. coli O157:H7 0, 8, and 60 days after hospitalization. These sera were used to examine the immune response to four different **EHEC** virulence factors: **Tir** (**translocated intimin receptor**, which is inserted into the host cell membrane), **intimin** (bacterial outer membrane **protein** which **binds** to **Tir**), EspA (secreted **protein** which forms filamentous structures on **EHEC** surface), and EspB (inserted into the host membrane and cytoplasm). The response to O157:H7 lipopolysaccharide was also examd. Sera were assayed against purified recombinant **proteins** using immunoblot anal. and by ELISA to det. the sera's titers to each of the antigens in all patients. We found that there was little reaction to EspA, EspB, and **intimin** in the acute-phase sera, although there was some reactivity to **Tir**. By day 8, titers of antibody to all four virulence factors were present in all patients, with a very strong response against **Tir** (up to a titer of 1:256,000), esp. in hemolytic-uremic syndrome patients, and lesser strong responses to the other three antigens. The titer to the antigens 60 days after hospitalization was decreased but was still highest for **Tir**. These results suggest that there is a strong immune response to **Tir**, and to a lesser extent to the other three virulence factors, following **EHEC** disease, indicating that these bacterial mols. are potential vaccine candidates for preventing **EHEC** disease. They also suggest that bacterial virulence factors that are inserted into host cells during infection by type III secretion systems (**Tir** or EspB) are still recognized by the host immune response.

REFERENCE COUNT: 2
REFERENCE(S): (1) Abe, A; J Exp Med 1998, V188, P1907 CAPLUS
(2) Bitzan, M; J Clin Microbiol 1992, V30, P1174 MEDLINE

L13 ANSWER 10 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:535365 CAPLUS
DOCUMENT NUMBER: 133:155433
TITLE: Inhibitors of **intimin** adhesion and tests for their screening

INVENTOR(S): Frankel, Gad Meir; Matthews, Stephen John; Hale, Christine Betty; Dougan, Gordon
PATENT ASSIGNEE(S): Imperial College Innovations Limited, UK
SOURCE: PCT Int. Appl., 96 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000045173	A1	20000803	WO 2000-GB254	20000131

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: GB 1999-1897 A 19990129
AB The invention relates to the provision of **polypeptides** which comprise or consist of the **Tir binding** domain of **intimin** and/or a **Tir**-independent eukaryotic cell **binding** activity and to the use of such **polypeptides** in methods of screening for agents which affect the **binding** of **intimin** to an eukaryotic cell, preferably an intestinal cell. Such inhibitors are useful in the prevention or treatment of bacterial infections, esp. those which cause diarrhea.

IT **287121-95-3**
RL: BOC (Biological occurrence); BPR (Biological process); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(amino acid sequence; inhibitors of **intimin** adhesion and tests for antidiarrheal screening)

REFERENCE COUNT: 15
REFERENCE(S):
(2) Armstrong; US 5858698 A 1999 CAPLUS
(3) Cravioto, A; JOURNAL OF INFECTIOUS DISEASES 1991, V163(6), P1247 CAPLUS
(4) Frankel, G; INFECTION AND IMMUNITY 1994, V62(5), P1835 CAPLUS
(5) Geyid, A; 1996, 7, CAPLUS
(6) Geyid, A; FEMS IMMUNOL MED MCROBIOL 1996, V14(1), P15 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 11 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:519996 CAPLUS
DOCUMENT NUMBER: 133:249419
TITLE: Expression of **intimin** .gamma. from **enterohemorrhagic** Escherichia **coli** in Citrobacter rodentium
AUTHOR(S): Hartland, Elizabeth L.; Huter, Veronika; Higgins, Lisa M.; Goncalves, Nathalie S.; Dougan, Gordon; Phillips, Alan D.; MacDonald, Thomas T.; Frankel, Gad
CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7 2AZ, UK
SOURCE: Infect. Immun. (2000), 68(8), 4637-4646
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The carboxy-terminal 280 amino acids (Int280) of the bacterial adhesion mol. **intimin** include the receptor-**binding** domain. At least 5 different types of Int280, designated .alpha., .beta., .gamma., .delta., and .epsilon., have been described based on sequence variation in this region. Importantly, the **intimin** types are assocd. with different evolutionary branches and contribute to distinct tissue tropism of **intimin**-pos. bacterial pathogens. This study describes how a strain of C. rodentium, which normally displays **intimin** .beta., was engineered to express **intimin** .gamma. from **enterohemorrhagic** E. **coli**. **Intimin** .gamma. bound to the **translocated intimin receptor** (**Tir**) from C. rodentium and had the ability to produce **attaching** and **effacing** lesions on HEp-2 cells. However, C. rodentium expressing **intimin** .gamma. could not colonize orally infected mice or induce mouse colonic hyperplasia. These results suggest that **intimin** may contribute to host specificity, possibly through its interaction with a receptor on the host cell surface.

REFERENCE COUNT: 33
REFERENCE(S):
(1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
(2) An, H; FEMS Microbiol Lett 1997, V148, P239 CAPLUS
(4) Batchelor, M; J Clin Microbiol 1999, V37, P3822 CAPLUS
(5) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS
(6) Donnenberg, M; Infect Immun 1991, V59, P4310

CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 12 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:497087 CAPLUS
DOCUMENT NUMBER: 133:219121
TITLE: Crystal structure of **enteropathogenic** Eschenchia **coli intimin** -receptor complex
AUTHOR(S): Luo, Yu; Frey, Elizabeth A.; Pfuetzner, Richard A.; Creagh, A. Louise; Knoechel, Derek G.; Haynes, Charles A.; Inlay, B. Brett; Strynadka, Natalie C. J.
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Nature (London) (2000), 405(6790), 1073-1077
CODEN: NATUAS; ISSN: 0028-0836
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Intimin** and its **translocated intimin receptor** (**Tir**) are bacterial **proteins** that mediate adhesion between mammalian cells and **attaching** and **effacing** (**A/E**) pathogens. **Enteropathogenic** Escherichia **coli** (**EPEC**) causes significant paediatric morbidity and mortality world-wide. A related **A/E** pathogen, **enterohaemorrhagic** E. **coli** (**EHEC**; 0157:H7) is one of the most important food-borne pathogens in North America, Europe and Japan. A unique and essential feature of **A/E** bacterial pathogens is the formation of actin-rich pedestals beneath the intimately adherent bacteria and localized destruction of the intestinal brush border. The bacterial outer membrane adhesin, **intimin**, is necessary for the prodn. of the **A/E** lesion and diarrhea. The **A/E** bacteria translocate their own receptor for **intimin**, **Tir**, into the membrane of mammalian cells using the type III secretion system. The translocated **Tir** triggers addnl. host signaling events and actin nucleation, which are essential for lesion formation. Here we describe the crystal structures of an **EPEC intimin** carboxyterminal fragment alone and in complex with the **EPEC Tir intimin-binding** domain, giving insight into the mol. mechanisms of adhesion of **A/E** pathogens.

REFERENCE COUNT: 30
REFERENCE(S): (3) Banner, D; J Mol Biol 1987, V196, P657
CAPLUS

(4) Betz, S; Biochemistry 1997, V36, P2450
CAPLUS
(6) Carson, M; J Mol Graphics 1986, V4, P121
CAPLUS
(8) DeVinney, R; Infect Immun 1999, V67, P2389
CAPLUS
(9) de La Fortelle, E; Methods Enzymol 1997,
V276, P472 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 13 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:472429 CAPLUS
DOCUMENT NUMBER: 133:219083
TITLE: Structural basis for recognition of the **translocated intimin receptor** (**Tir**) by **intimin** from **enteropathogenic** Escherichia **coli**
AUTHOR(S): Batchelor, Miranda; Prasannan, Sunil; Daniell, Sarah; Reece, Stephen; Connerton, Ian; Bloomberg, Graham; Dougan, Gordon; Frankel, Gad; Matthews, Stephen
CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7 2AZ, UK
SOURCE: EMBO J. (2000), 19(11), 2452-2464
CODEN: EMJODG; ISSN: 0261-4189
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Intimin** is a bacterial adhesion mol. involved in intimate attachment of **enteropathogenic** and **enterohaemorrhagic** Escherichia **coli** to mammalian host cells. **Intimin** targets the **translocated intimin receptor** (**Tir**), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study we localized the **Tir-binding** region of **intimin** to the C-terminal 190 amino acids (Int190). We have also detd. the region's high-resoln. soln. structure, which comprises an Ig domain that is intimately coupled to a novel C-type lectin domain. This fragment, which is necessary and sufficient for **Tir** interaction, defines a new super domain in **intimin** that exhibits striking structural similarity to the integrin-**binding** domain of the Yersinia invasin and C-type lectin families. The extracellular portion of **intimin** comprises an articulated rod of Ig domains extending from the bacterium surface, conveying a highly accessible "adhesive tip" to the target cell. The interpretation of

NMR-titrn. and mutagenesis data has enabled us to identify, for the first time, the **binding** site for **Tir**, which is located at the extremity of the Int190 moiety.

REFERENCE COUNT: 59

REFERENCE(S):
(1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
(2) Bax, A; Magn Res 1990, V88, P425 CAPLUS
(3) Boyington, J; Immunity 1999, V10, P75 CAPLUS
(5) Cornilescu, G; J Biomol NMR 1999, V13, P289 CAPLUS
(6) de Grado, M; Cell Microbiol 1999, V1, P7 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 14 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:438489 CAPLUS
DOCUMENT NUMBER: 133:161648
TITLE: Mechanical fractionation reveals structural requirements for **enteropathogenic** Escherichia **coli Tir** insertion into host membranes
AUTHOR(S): Gauthier, Annick; De Grado, Myriam; Finlay, B. Brett
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology and Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Infect. Immun. (2000), 68(7), 4344-4348
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) inserts its receptor for intimate adherence (**Tir**) into host cell membranes by using a type III secretion system. Detergents are frequently used to fractionate infected host cells to investigate bacterial **protein** delivery into mammalian cells. In this study, the Triton X-100-sol. membrane fraction from **EPEC**-infected HeLa cells was contaminated with bacterial **proteins**. Therefore, a mech. method of cell lysis and ultracentrifugation to fractionate infected HeLa cells was applied to investigate the biol. and biochem. of **Tir** delivery and translocation. This method demonstrates that the translocation of **Tir** into the host cell membrane requires its transmembrane domains, but not tyrosine phosphorylation or **binding** to **Tir**'s ligand, **intimin**.

REFERENCE COUNT: 31

REFERENCE(S):
(1) Abe, A; Mol Microbiol 1999, V33, P1162 CAPLUS

(2) Bauer, M; Trends Cell Biol 2000, V10, P25 CAPLUS
(3) Collazo, C; Mol Microbiol 1997, V24, P747 CAPLUS
(4) DeVinney, R; Cell Mol Life Sci 1999, V55, P961 CAPLUS
(5) DeVinney, R; Infect Immun 1999, V67, P2389 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 15 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:431539 CAPLUS
DOCUMENT NUMBER: 133:174359
TITLE: **Intimin** from **enteropathogenic** Escherichia **coli** mediates remodelling of the eukaryotic cell surface
AUTHOR(S): Phillips, Alan D.; Giron, Jorge; Hicks, Susan; Dougan, Gordon; Frankel, Gad
CORPORATE SOURCE: University Department of Paediatric Gastroenterology, Royal Free Hospital, London, NW3 2QG, UK
SOURCE: Microbiology (Reading, U. K.) (2000), 146(6), 1333-1344
CODEN: MROBEO; ISSN: 1350-0872
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Adhesion to cultured epithelial cells by **enteropathogenic** Escherichia **coli** (**EPEC**) is assocd. with extensive rearrangement of the host cell cytoskeleton. Evidence has been presented that **EPEC** adhesion is assocd. with activation of signal transduction pathways leading to prodn. of a characteristic histopathol. feature known as the **attaching** and **effacing** (**A/E**) lesion. **A** /**E** lesion formation requires **intimin**, an **EPEC** adhesion mol. and several **EPEC** secreted **proteins** (EspA, B, D and **Tir**) involved in cell signalling and **protein** translocation. In this study it is shown that HEp-2 cells respond during the early stages of infection with two wild-type **EPEC** strains (B171 and E2348/69) by producing microvillus-like processes (MLP) at the site of initial bacterial adherence. **Intimin** appears to play a key role in MLP elongation. At later stages of infection with these wild-type **EPEC** strains, when **A/E** lesions have formed, the MLP were reduced in no. and length to appear as at time zero, and the cell surface in the vicinity of bacterial clusters appeared unaffected. In contrast, infection with EspA- or EspB-neg., but **intimin**-pos., **EPEC**

strains (UMD872 and UMD864, resp.) resulted in enhanced MLP proliferation and formation of cage-like structures engulfing the bacteria. Inoculating HEp-2 cells with **intimin**-coated latex spheres induced similar cage-like structures. Caco-2 cells did not show **intimin**-induced microvillus elongation in response to **EPEC** infection, although microvillus effacement and redn. in no. occurred. Similar phenomena appeared on B171 and E2348/69 infection of pediatric intestine using in vitro organ culture, i.e. elongated microvilli were seen in assocn. with small colonies and at the periphery of large localized colonies, along with evidence of microvillus breakdown and debris in the colony center. These results show that **intimin** activates signal transduction pathways involved in the remodelling of the eukaryotic cell surface, probably via **binding** to a receptor encoded by the host cell.

REFERENCE COUNT: 44
REFERENCE(S):
(1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
(2) Bain, C; Infect Immun 1998, V66, P3900 CAPLUS
(4) Ben-Ami, G; Infect Immun 1998, V66, P1755 CAPLUS
(6) Donnenberg, M; Infect Immun 1991, V59, P4310 CAPLUS
(7) Donnenberg, M; J Bacteriol 1993, V175, P4670 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 16 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:421954 CAPLUS
DOCUMENT NUMBER: 133:147284
TITLE: **Enteropathogenic E. coli translocated intimin receptor, Tir**, interacts directly with .alpha.-actinin
AUTHOR(S): Goosney, Danika L.; DeVinney, Rebekah; Pfuetzner, Richard A.; Frey, Elizabeth A.; Strynadka, Natalie C.; Finlay, B. Brett
CORPORATE SOURCE: Biotechnology Laboratory, The Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Curr. Biol. (2000), 10(12), 735-738
CODEN: CUBLE2; ISSN: 0960-9822
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Enteropathogenic** Escherichia **coli** (**EPEC**) triggers a dramatic rearrangement of the host epithelial cell

actin cytoskeleton to form an **attaching** and **effacing** lesion, or pedestal. The pathogen remains attached extracellularly to the host cell through the pedestal for the duration of the infection. At the tip of the pedestal is a bacterial **protein**, **Tir**, which is secreted from the bacterium into the host cell plasma membrane, where it functions as the receptor for an **EPEC** outer membrane **protein** , **intimin** [1]. Delivery of **Tir** to the host cell results in its tyrosine phosphorylation, followed by **Tir-intimin binding**. **Tir** is believed to anchor **EPEC** firmly to the host cell, although its direct linkage to the cytoskeleton is unknown. Here, we show that **Tir** directly **binds** the cytoskeletal **protein** .alpha.-actinin. .alpha.-Actinin is recruited to the pedestal in a **Tir**-dependent manner and colocalizes with **Tir** in infected host cells. **Binding** is mediated through the amino terminus of **Tir**. Recruitment of .alpha.-actinin occurs independently of **Tir** tyrosine phosphorylation. Recruitment of actin, VASP, and N-WASP, however, is abolished in the absence of this tyrosine phosphorylation. These results suggest that **Tir** plays at least three roles in the host cell during infection: **binding intimin** on **EPEC**; mediating a stable anchor with .alpha.-actinin through its amino terminus in a phosphotyrosine-independent manner; and recruiting addnl. cytoskeletal **proteins** at the carboxyl terminus in a phosphotyrosine-dependent manner. These findings demonstrate the first known direct linkage between extracellular **EPEC**, through the transmembrane **protein** **Tir**, to the host cell actin cytoskeleton via .alpha.-actinin.

REFERENCE COUNT: 14

REFERENCE(S):

(1) Abe, A; Mol Microbiol 1999, V33, P1162 CAPLUS

(2) deGrado, M; Cellular Microbiol 1999, V1, P7 CAPLUS

(3) Dramsi, S; Annu Rev Cell Dev Biol 1998, V14, P137 CAPLUS

(4) Frankel, G; Infect Immun 1995, V63, P4323 CAPLUS

(5) Frankel, G; J Biol Chem 1996, V271, P20359 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 17 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:298095 CAPLUS

DOCUMENT NUMBER: 133:71203

TITLE: Identification of the **intimin-binding** domain of **Tir** of

enteropathogenic Escherichia **coli**

AUTHOR(S): De Grado, Myriam; Abe, Akio; Gauthier, Annick; Steele-Mortimer, Olivia; DeVinney, Rebekah; Finlay, B. Brett

CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Cell. Microbiol. (1999), 1(1), 7-17
CODEN: CEMIF5; ISSN: 1462-5814

PUBLISHER: Blackwell Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) attaches intimately to mammalian cells via a bacterial outer membrane adhesion mol., **intimin**, and its receptor in the host cell membrane, **Tir**. **Tir** is a bacterial **protein** translocated into the host cell membrane and tyrosine phosphorylated after insertion. **Tir-intimin binding** induces organized actin polymn. beneath the adherent bacteria, resulting in the formation of pedestal-like structures. A series of **Tir** deletion derivs. were constructed to analyze which **Tir** domains are involved in **intimin binding**. We have localized the **intimin-binding** domain (IBD) of **Tir** using a yeast two-hybrid system and a gel-overlay approach to a region of 109 amino acids that is predicted to be exposed on the surface of the plasma membrane. A truncated **Tir** **protein** lacking this domain was translocated to the host cell membrane and tyrosine phosphorylated, but failed to **bind intimin** or to induce either actin polymn. or **Tir** accumulation beneath the bacteria. These results indicate that only a small region of **Tir** is needed to **bind intimin** and support the predicted topol. for **Tir**, with both N- and C-terminal regions in the mammalian cell cytosol. They also confirm that **Tir-intimin** interactions are needed for cytoskeletal organization. We have also identified N-terminal regions involved in **Tir** stability and **Tir** secretion to the media.

REFERENCE COUNT: 27

REFERENCE(S):

(1) DeVinney, R; Infect Immun 1999, V67, P2389 CAPLUS

(3) Donnenberg, M; Infect Immun 1991, V59, P4310 CAPLUS

(4) Donnenberg, M; J Bacteriol 1993, V175, P4670 CAPLUS

(5) Donnenberg, M; J Clin Invest 1993, V92, P1412 CAPLUS

(6) Elliott, S; Mol Microbiol 1998, V28, P1

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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 18 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:279346 CAPLUS
DOCUMENT NUMBER: 133:295077
TITLE: Human colostrum and serum contain antibodies reactive to the **intimin-binding** region of the **enteropathogenic** Escherichia **coli translocated intimin receptor**
AUTHOR(S): Sanches, Marcela Imperio; Keller, Rogeria; Hartland, Elizabeth L.; Figueiredo, Dayse M. M.; Batchelor, Miranda; Martinez, Marina B.; Dougan, Gordon; Careiro-Sampaio, Magda M. S.; Frankel, Gad; Trabulsi, Luiz R.
CORPORATE SOURCE: Departamento de Microbiologia, Instituto de Ciencias Biomedicas, Departamento de Immunologia, ICB III and Faculdade de Ciencias Farmaceutica, Departamento de Analises Clinicas e Toxicologicas Universidade de Sao Paulo, Sao Paulo, Brazil
SOURCE: J. Pediatr. Gastroenterol. Nutr. (2000), 30(1), 73-77
CODEN: JPGND6; ISSN: 0277-2116
PUBLISHER: Lippincott Williams & Wilkins
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Background: In Brazil, **enteropathogenic** Escherichia **coli** (**EPEC**) diarrhoea is endemic in young infants. A characteristic feature of **EPEC** adhesion to host cells is intimate attachment leading to the formation of distinctive "**attaching** and **effacing**" (**A/E**) lesions on mammalian cells. Two genes directly involved in intimate adhesion, eae and **tir**, encode the adhesion mol. **intimin** and its translocated receptor **Tir**, resp. The **intimin-binding** domain of **Tir** was recently mapped to the middle part of the **polypeptide** (**Tir**-M), and the amino (**Tir**-N) and carboxy (**Tir**-C) termini were found to be located within infected host cells. Recently, it was shown that colostrum samples from mothers living in Sao Paulo contain IgA-class antibodies reactive with a no. of **proteins** assocd. with **EPEC** virulence. It has also been shown that patients infected with verocytotoxin-producing E. coli O157 can produce antibodies to **Tir**. In the current study antibody responses to the different **Tir** domains were analyzed in sera and colostrum samples collected in an

EPEC-endemic area of Brazil. Methods: Recombinant **Tir**, **Tir**-N, **Tir**-M, and **Tir**-C were expressed as His-tagged **protein** in E. coli BL21a and purified on nickel columns. Western blot anal. was used to investigate colostrum IgA- and serum IgG-class antibodies reactive with the **Tir** fragments. Results: Anti-**Tir** IgG antibodies were detected in the serum of children, with (63%) or without (50%) diarrhoea. Anti-**Tir** IgA-class antibodies were detected in all the colostrum pools tested. With the use of both serum IgG- and colostrum IgA-class antibodies, an immunodominant domain of the **Tir-polypeptide**, **Tir** M, was identified. Conclusion: The **intimin-binding** region of **Tir** (**Tir**-M) is the immunodominant region of the **polypeptide** in humans. Both serum IgG-class and colostrum IgA-class antibodies reacted predominantly with the **Tir**-M domain.

REFERENCE COUNT: 21

REFERENCE(S):
(1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
(3) Camara, L; Int Arch Allergy Immunol 1994, V103, P307 CAPLUS
(4) Elliott, S; Mol Microbiol 1998, V28, P1 CAPLUS
(5) Frankel, G; Infect Immun 1994, V62, P1835 CAPLUS
(6) Frankel, G; Infect Immun 1996, V64, P5315 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 19 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:51065 CAPLUS

DOCUMENT NUMBER: 133:16094

TITLE: Antibody response of patients infected with verocytotoxin-producing Escherichia coli to **protein** antigens encoded on the LEE locus

AUTHOR(S): Jenkins, C.; Chart, H.; Smith, H. R.; Hartland, E. L.; Batchelor, M.; Delahay, R. M.; Dougan, G.; Frankel, G.

CORPORATE SOURCE: Laboratory of Enteric Pathogens, Central Public Health Laboratory, London, NW9 5HT, UK

SOURCE: J. Med. Microbiol. (2000), 49(1), 97-101
CODEN: JMMIAV; ISSN: 0022-2615

PUBLISHER: Lippincott Williams & Wilkins

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Sera from patients infected with verocytotoxin-producing Escherichia coli (VTEC) O157, from patients with antibodies to E. coli O157

lipopolysaccharide (LPS) and from healthy controls were examd. for antibodies to **proteins** involved in expressing the **attaching** and **effacing** phenotype. After SDS-PAGE, purified recombinant **intimin**, EspA-filament structural **protein**, translocated **protein** EspB and three sep. domains of the **translocated intimin receptor** (**Tir**) were tested for reaction with patients' sera by immunoblotting. An ELISA was also used to detect antibodies to **intimin** in sera from E. coli O157 LPS antibody-pos. individuals. Seven of nine culture-pos. patients and one control patient had antibodies to EspA. Five of these patients and two controls had serum antibodies to the **intimin-binding** region of **Tir**, whereas none of the sera contained antibodies **binding** to either of the intracellular domains of **Tir**. By immunoblotting, 10 of 14 culture-pos. patients had antibodies to the conserved region of **intimin**, eight of whom were infected with E. coli O157 phage type 2. Thirty six of 60 sera from culture-neg. but E. coli O157 LPS antibody-pos. patients had antibodies to **intimin** as detd. by ELISA. The secreted **proteins** are expressed in vivo during infection and are considered as pathogenic markers. Antibodies to these **proteins** may from the basis of a serodiagnostic test for the detection of patients infected with VTEC which carry the locus for the enterocyte effacement pathogenicity island and provide an adjunct test to the established serol. tests based on VTEC LPS.

REFERENCE COUNT: 16

REFERENCE(S):
(1) Adu-Bobie, J; Infect Immun 1998, V66, P5643 CAPLUS
(2) Chart, H; Epidemiol Infect 1998, V120, P239 CAPLUS
(3) Chart, H; Lancet 1998, V352, P371 CAPLUS
(6) Frankel, G; Infect Immun 1994, V62, P1835 CAPLUS
(7) Frankel, G; Mol Microbiol 1998, V30, P911 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 20 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:26661 CAPLUS

DOCUMENT NUMBER: 132:176540

TITLE: Hierarchy in the expression of the locus of enterocyte effacement genes of **enteropathogenic** Escherichia **coli**

AUTHOR(S): Friedberg, Devorah; Umanski, Tatiana; Fang, Yuan; Rosenshine, Ilan

CORPORATE SOURCE: Departments of Molecular Genetics and

Biotechnology, Faculty of Medicine, The Hebrew University, Jerusalem, 91120, Israel
SOURCE: Mol. Microbiol. (1999), 34(5), 941-952
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Enteropathogenic** Escherichia **coli** (**EPEC**) elicit changes in host cell morphol. and cause actin rearrangement, a phenotype that has commonly been referred to as **attaching/effacing** (AE) lesions. The ability of **EPEC** to induce AE lesions is dependent upon a type III **protein** secretion/translocation system that is encoded by genes clustered in a 35.6 kb DNA segment, named the locus of enterocyte effacement (LEE). The authors used transcriptional fusions between the green fluorescent **protein** (gfp) reporter gene and LEE genes rorf2, orf3, orf5, escJ, escV and eae, together with immunoblot anal. with antibodies against **Tir** , **intimin**, EspB and EspF, to analyze the genetic regulation of the LEE. The expression of all these LEE genes was strictly dependent upon the presence of a functional integration host factor (IHF). IHF **binds** specifically upstream from the ler (orf1) promoter and appears to activate expression of ler, orf3, orf5 and rorf2 directly. The ler-encoded Ler **protein** was involved in activating the expression of escJ, escV, **tir** , eae, espB and espF. Expression of both IHF and Ler was needed to elicit actin rearrangement assocd. with AE lesions. In conclusion, IHF directly activates the expression of the ler and rorf2 transcriptional units, and Ler in turn mediates the expression of the other LEE genes.
REFERENCE COUNT: 27
REFERENCE(S):
(2) Brosius, J; Proc Natl Acad Sci USA 1984, V81, P6929 CAPLUS
(3) Cormack, B; Gene 1996, V173, P33 CAPLUS
(4) Craig, N; Cell 1984, V39, P707 CAPLUS
(5) Donnenberg, M; Infect Immun 1991, V59, P4310 CAPLUS
(6) Elliott, S; Mol Microbiol 1998, V28, P1 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 21 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:675935 CAPLUS
DOCUMENT NUMBER: 132:20883
TITLE: The **Tir-binding** region of **enterohemorrhagic** Escherichia **coli intimin** is sufficient to trigger actin condensation after

bacterial-induced host cell signalling

AUTHOR(S): Liu, Hui; Magoun, Loranne; Luperchio, Steve; Schauer, David B.; Leong, John M.

CORPORATE SOURCE: Department of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester, MA, 01655, USA

SOURCE: Mol. Microbiol. (1999), 34(1), 67-81
CODEN: MOMIEE; ISSN: 0950-382X

PUBLISHER: Blackwell Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Enterohemorrhagic** Escherichia **coli** (**EHEC**) has emerged as an important agent of diarrheal disease. Attachment to host cells, an essential step during intestinal colonization by **EHEC**, is assocd. with the formation of a highly organized cytoskeletal structure contg. filamentous actin, termed an **attaching** and **effacing** (**A/E**) lesion, directly beneath bound bacteria. The outer membrane **protein intimin** is required for the formation of this structure, as is **Tir**, a bacterial **protein** that is translocated into the host cell and is thought to function as a receptor for **intimin**. To understand **intimin** function better, the authors fused **EHEC intimin** to a homologous **protein**, Yersinia pseudotuberculosis invasin, or to maltose-**binding protein**. The N-terminal 539 amino acids of **intimin** were sufficient to promote outer membrane localization of the C-terminus of invasin and, conversely, the N-terminal 489 amino acids of invasin were sufficient to promote the localization of the C-terminus of **intimin**. The C-terminal 181 residues of **intimin** were sufficient to **bind** mammalian cells that had been preinfected with an **enteropathogenic** E. **coli** strain that expresses **Tir** but not **intimin**. **Binding** of **intimin** derivs. to preinfected cells correlated with **binding** to recombinant **Tir protein**. Finally, the 181-residue minimal **Tir-binding** region of **intimin**, when purified and immobilized on latex beads, was sufficient to trigger **A/E** lesions on preinfected mammalian cells.

REFERENCE COUNT: 60

REFERENCE(S):
(3) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS
(4) Dersch, P; EMBO J 1999, V18, P1199 CAPLUS
(5) Donnenberg, M; Infect Immun 1991, V59, P4310 CAPLUS
(6) Donnenberg, M; Infect Immun 1992, V60, P3953 CAPLUS
(8) Foubister, V; J Exp Med 1994, V179, P993

CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 22 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:632014 CAPLUS
DOCUMENT NUMBER: 131:333663
TITLE: Identification of CesT, a chaperone for the type III secretion of **Tir** in **enteropathogenic** Escherichia **coli**
AUTHOR(S): Elliott, Simon J.; Hutcheson, Steven W.; Dubois, Maria S.; Mellies, Jay L.; Wainwright, Leslie A.; Batchelor, Miranda; Frankel, Gad; Knutton, Stuart; Kaper, James B.
CORPORATE SOURCE: Center for Vaccine Development and Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, 21201, USA
SOURCE: Mol. Microbiol. (1999), 33(6), 1176-1189
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The locus of enterocyte effacement of **enteropathogenic** Escherichia **coli** encodes a type III secretion system, an outer membrane **protein** adhesin (**intimin**, the product of eae) and **Tir**, a translocated **protein** that becomes a host cell receptor for **intimin**. Many type III secreted **proteins** require chaperones, which function to stabilize **proteins**, prevent inappropriate **protein-protein** interactions and aid in secretion. An open reading frame located between **tir** and eae, previously named orfU, was predicted to encode a **protein** with partial similarity to the Yersinia SycH chaperone. The authors examd. the potential of the orfU gene product to serve as a chaperone for **Tir**. The orfU gene encoded a 15 kDa cytoplasmic **protein** that specifically interacted with **Tir** as demonstrated by the yeast two-hybrid assay, column **binding** and coimmunopptn. expts. An orfU mutant was defective in **attaching-effacing** lesion formation and **Tir** secretion, but was unaffected in expression of other virulence factors. OrfU appeared to stabilize **Tir** levels in the cytoplasm, but was not absolutely necessary for secretion of **Tir**. Based upon the phys. similarities, phenotypic characteristics and the demonstrated interaction with **Tir**, orfU is redesignated as cesT for the chaperone for E. coli secretion of **Tir**.
REFERENCE COUNT: 47

REFERENCE(S): (1) Abe, A; Mol Microbiol 1999, V33 CAPLUS
(2) Anderson, D; Science 1997, V278, P1140 CAPLUS
(5) Cheng, L; Mol Microbiol 1997, V24, P757 CAPLUS
(6) Clark, K; Proc Natl Acad Sci USA 1998, V95, P5401 CAPLUS
(8) Cornelis, G; Microbiol Mol Biol Rev 1998, V62, P1315 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 23 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:632013 CAPLUS
DOCUMENT NUMBER: 131:333662
TITLE: **Enteropathogenic** Escherichia **coli translocated intimin receptor, Tir**, requires a specific chaperone for stable secretion
AUTHOR(S): Abe, Akio; De Grado, Myriam; Pfuetzner, Richard A.; Sanchez-SanMartin, Claudia; DeVinney, Rebekah; Puente, Jose Luis; Strynadka, Natalie C. J.; Finlay, B. Brett
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Mol. Microbiol. (1999), 33(6), 1162-1175
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Enteropathogenic** Escherichia **coli** (**EPEC**) secretes several Esps (E. coli-secreted **proteins**) that are required for full virulence. Insertion of the bacterial **protein Tir** into the host epithelial cell membrane is facilitated by a type III secretion app., and at least EspA and EspB are required for **Tir** translocation. An **EPEC** outer membrane **protein, intimin**, interacts with **Tir** on the host membrane to establish intimate attachment and formation of a pedestal-like structure. In this study, we identified a **Tir** chaperone, CesT, whose gene is located between **tir** and eae (which encodes **intimin**). A mutation in cesT abolished **Tir** secretion into culture supernatants and significantly decreased the amt. of **Tir** in the bacterial cytoplasm. In contrast, this mutation did not affect the secretion of the Esp **proteins**. The level of **tir** mRNA was not affected by the cesT mutation, indicating that CesT acts at the post-transcriptional level. The cesT mutant could not induce host cytoskeletal rearrangements, and displayed the

same phenotype as the **tir** mutant. Gel overlay and GST pulldown assays demonstrated that CesT specifically interacts with **Tir**, but not with other Esp **proteins**. Furthermore, by using a series of **Tir** deletion derivs., we detd. that the CesT **binding** domain is located within the first 100 amino-terminal residues of **Tir**, and that the pool of **Tir** in the bacterial cytoplasm was greatly reduced when this domain was disrupted. Interestingly, this domain was not sufficient for **Tir** secretion, and at least the first 200 residues of **Tir** were required for efficient secretion. Gel filtration studies showed that **Tir**-CesT forms a large multimeric complex. Collectively, these results indicate that CesT is a **Tir** chaperone that may act as an anti-degrdn. factor by specifically **binding** to its amino-terminus, forming a multimeric stabilized complex.

REFERENCE COUNT: 57

REFERENCE(S):
(1) Abe, A; J Exp Med 1998, V188, P1907 CAPLUS
(2) An, H; FEMS Microbiol Lett 1997, V148, P239 CAPLUS
(3) Anderson, D; Science 1997, V278, P1140 CAPLUS
(4) Beaudry, M; J Clin Microbiol 1996, V34, P144 CAPLUS
(6) Cheng, L; Mol Microbiol 1997, V24, P757 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 24 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:569268 CAPLUS

DOCUMENT NUMBER: 131:307586

TITLE: A novel chromosomal locus of **enteropathogenic** Escherichia **coli** (**EPEC**), which encodes a bfpT-regulated chaperone-like **protein**, trcA, involved in microcolony formation by **EPEC**

AUTHOR(S): Tobe, Toru; Tatsuno, Ichiro; Katayama, Eisaku; Wu, Cheng-Yen; Schoolnik, Gary K.; Sasakawa, Chihiro

CORPORATE SOURCE: Department of Bacteriology, University of Tokyo, Tokyo, 108-0071, Japan

SOURCE: Mol. Microbiol. (1999), 33(4), 741-752
CODEN: MOMIEE; ISSN: 0950-382X

PUBLISHER: Blackwell Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The bfpTVW operon, also known as the per operon, of **enteropathogenic** Escherichia **coli** (**EPEC**)

is required for the transcriptional activation of the bfp operon, which encodes the major subunit and assembly machinery of bundle-forming pili (BFP). An immobilized T7-tagged BfpT fusion **protein** that **binds** specifically to upstream promoter sequences of bfpA and eae was used to "fish out" from a promoter library other **EPEC** chromosomal fragments that are bound by the BfpT **protein**. After screening for promoters exhibiting bfpTVW-dependent expression, one was identified that was pos. regulated by bfpTVW and that is not present in the chromosomes of two non-virulent E. coli lab. strains, DH5.alpha. and HB101. Further anal. of this pos. regulated promoter in **EPEC** showed that it resided within a 4.9 kb sequence that is not present in E. coli K12. This locus, located downstream of the potB gene, was found to contain four open reading frames (ORFs): bfpTVW-activated promoter was localized upstream of ORF1. An ORF1 knockout mutant produced less of the BFP structural subunit (BfpA) and formed smaller than normal adherent microcolonies on cultured epithelial cells; however, this mutation did not affect bfp transcription. An ORF1-His6 fusion **protein** specifically bound the preprocessed and mature forms of the BfpA **protein** and thus appears to stabilize the former within the cytoplasmic compartment. ORF1 therefore is a newly isolated **EPEC** chromosomal gene that encodes a chaperone-like **protein** involved in the prodn. of BFP. Hence, ORF1 was designated trcA (bfpT-regulated chaperone-like **protein** gene). The TrcA **protein** also specifically bound 39 kDa and **90 kDa proteins** that are expressed by **EPEC** but not by E. coli K12. The **90 kDa protein** was revealed to be **intimin**, a **protein** product of the eae gene, which is required for the **EPEC attaching/effacing** phenotype, suggesting a direct interaction of TrcA with **intimin** in the cytoplasmic compartment.

REFERENCE COUNT: 46

REFERENCE(S):
(1) Allaoui, A; Mol Microbiol 1992, V6, P1605 CAPLUS
(3) Bieber, D; Science 1998, V280, P2114 CAPLUS
(4) Bilge, S; Infect Immun 1996, V64, P4795 CAPLUS
(5) Boyer, H; J Mol Biol 1969, V41, P459 CAPLUS
(6) Brosius, J; Gene 1984, V27, P151 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 25 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:326051 CAPLUS

DOCUMENT NUMBER: 130:333761

TITLE: Pathogenic Escherichia coli **intimin** receptor **Tir** and gene **tir**

and methods for detecting gene **tir** or **Tir protein** and for drug screening

INVENTOR(S): Finlay, B. Brett; Kenny, Brendan; Devinney, Rebekah; Stein, Marcus

PATENT ASSIGNEE(S): University of British Columbia, Can.

SOURCE: PCT Int. Appl., 91 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9924576	A1	19990520	WO 1998-CA1042	19981110
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9911373	A1	19990531	AU 1999-11373	19981110
EP 1029054	A1	20000823	EP 1998-954076	19981110
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: US 1997-65130 P 19971112
WO 1998-CA1042 W 19981110

AB A **polypeptide**, called **Tir** (for **translocated intimin receptor**), which is secreted by **attaching** and **effacing** pathogens, such as the **enteropathogenic** (**EPEC**) and **enterohemorrhagic** (**EHEC**) E. **coli** is disclosed. These bacterial pathogens insert their own receptors into mammalian cell surfaces, to which the bacterial pathogen then adheres to trigger addnl. host signaling events and actin nucleation. Diagnosis of disease caused by pathogenic E. coli can be performed by the use of antibodies which **bind** to **Tir** to detect the **protein** or the use of nucleic acid probes for detection of nucleic acids encoding **Tir polypeptide**. Isolated nucleic acid sequences encoding **Tir polypeptide**, **Tir peptides**, a recombinant method for producing recombinant **Tir**, antibodies which **bind** to **Tir**, and a kit for the detection of **Tir**-producing E. coli are provided. A method

of immunizing a host with **Tir** to induce a protective immune response to **Tir** or a second **polypeptide** of interest is also provided. A method for screening for compds. which interfere with the **binding** of bacterial pathogens to their receptors is further provided. Thus, **protein Hp90**, previously believed to be a host membrane **protein**, has been identified as an **EHEC**- or **EPEC**-secreted **protein** which acts as an **intimin** receptor. **Proteins** encoded by the espA and espB genes were necessary for delivery of **Tir** to the host membrane.

IT **200662-09-5P 224307-15-7P**
RL: ANT (Analyte); BOC (Biological occurrence); BPN (Biosynthetic preparation); BPR (Biological process); PRP (Properties); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); PROC (Process)
(amino acid sequence; pathogenic Escherichia coli **intimin** receptor **Tir** and gene **tir** and methods for detecting gene **tir** or **Tir protein** and for drug screening)

REFERENCE COUNT: 6
REFERENCE(S):
(1) Deibel, C; Molecular Microbiology 1998, V28(3), P463 CAPLUS
(2) Kenny, B; Cell 1997, V91, P511 CAPLUS
(3) Kenny, B; Infection and Immunity 1997, V65(7), P2528 CAPLUS
(4) Paton, A; Database EMBL - EMPRO 1998
(5) Paton, A; Infection and Immunity 1998, V66(11), P5580 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 26 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:291203 CAPLUS
DOCUMENT NUMBER: 131:85390
TITLE: **Enterohemorrhagic** Escherichia **coli** O157:H7 produces **Tir**, which is translocated to the host cell membrane but is not tyrosine phosphorylated
AUTHOR(S): DeVinney, Rebekah; Stein, Markus; Reinscheid, Dieter; Abe, Akio; Ruschkowski, Sharon; Finlay, B. Brett
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1ZA, Can.
SOURCE: Infect. Immun. (1999), 67(5), 2389-2398
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Intimate attachment to the host cell leading to the formation of **attaching** and **effacing** (**A/E**) lesions is an essential feature of **enterohemorrhagic** Escherichia **coli** (**EHEC**) O157:H7 pathogenesis. In a related pathogen, **enteropathogenic** E. **coli** (**EPEC**), this activity is dependent upon translocation of the **intimin** receptor, **Tir**, which becomes tyrosine phosphorylated within the host cell membrane. In contrast, the accumulation of tyrosine-phosphorylated **proteins** beneath adherent **EHEC** bacteria does not occur, leading to questions about whether **EHEC** uses a **Tir**-based mechanism for adherence and **A/E** lesion formation. In this report, we demonstrate that **EHEC** produces a functional **Tir** that is inserted into host cell membranes, where it serves as an **intimin** receptor. However, unlike in **EPEC**, in **EHEC Tir** is not tyrosine phosphorylated yet plays a key role in both bacterial adherence to epithelial cells and pedestal formation. **EHEC**, but not **EPEC**, was unable to synthesize **Tir** in Luria-Bertani medium but was able to secrete **Tir** into M9 medium, suggesting that **Tir** synthesis and secretion may be regulated differently in these two pathogens. **EHEC Tir** and **EPEC Tir** both **bind intimin** and focus cytoskeletal rearrangements, indicating that tyrosine phosphorylation is not needed for pedestal formation. **EHEC** and **EPEC intimins** are functionally interchangeable, but **EHEC Tir** shows a much greater affinity for **EHEC intimin** than for **EPEC intimin**. These findings highlight some of the differences and similarities between **EHEC** and **EPEC** virulence mechanisms, which can be exploited to further define the mol. basis of pedestal formation.

IT **212262-89-0**
RL: PRP (Properties)
(amino acid sequence; **enterohemorrhagic** Escherichia **coli** O157:H7 produces **Tir**, which is translocated to host cell membrane but is not tyrosine phosphorylated)

REFERENCE COUNT: 45
REFERENCE(S):
(1) Abe, A; Infect Immun 1997, V65, P3547 CAPLUS
(4) Bieber, D; Science 1998, V280, P2114 CAPLUS
(5) Brunder, W; V Mol:Microbiol 1997, V24, P767 CAPLUS
(6) Dean-Nystrom, E; Infect Immun 1998, V66, P4560 CAPLUS
(7) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, [illegible] TOXLIT, TOXLINE' ENTERED AT 12:00:10 ON 28 SEP 2001)

- Author(s)

L11	1156	S FINLAY B?/AU
L12	350	S KENNY B?/AU
L13	62	S (DE VINNEY R? OR DEVINNEY R?)/AU
L14	4379	S STEIN M?/AU
L15	8	S L11 AND L12 AND L13 AND L14
L16	108	S L11 AND (L12 OR L13 OR L14)
L17	29	S L12 AND (L13 OR L14)
L18	12	S L13 AND L14
L19	5798	S L11 OR L12 OR L13 OR L14
L20	110	S (L16 OR L19) AND L4
L21	7	S L20 AND (PURE OR PURIF?)
[illegible]	39	S L15 OR L17 OR L18 OR L21

[illegible] REMOVED)

Searcher : Shears 308-4994

L23 ANSWER 1 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
ACCESSION NUMBER: 2000:603715 CAPLUS
DOCUMENT NUMBER: 133:280269
TITLE: Human response to Escherichia coli O157:H7 infection: antibodies to secreted virulence factors
AUTHOR(S): Li, Yuling; Frey, Elizabeth; Mackenzie, Andrew M. R.; **Finlay, B. Brett**
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Infect. Immun. (2000), 68(9), 5090-5095
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Vaccination has been proposed for the prevention of disease due to enterohemorrhagic Escherichia coli (EHEC), but the immune response following human infection, including the choice of potential antigens, has not been well characterized. To study this, sera were obtained from five pediatric patients with acute diarrhea caused by E. coli O157:H7 0, 8, and 60 days after hospitalization. These sera were used to examine the immune response to four different EHEC virulence factors: **Tir** (**translocated intimin receptor**, which is inserted into the host cell membrane), intimin (bacterial outer membrane protein which binds to **Tir**), EspA (secreted protein which forms filamentous structures on EHEC surface), and EspB (inserted into the host membrane and cytoplasm). The response to O157:H7 lipopolysaccharide was also examd. Sera were assayed against **purified** recombinant proteins using immunoblot anal. and by ELISA to det. the sera's titers to each of the antigens in all patients. We found that there was little reaction to EspA, EspB, and intimin in the acute-phase sera, although there was some reactivity to **Tir**. By day 8, titers of antibody to all four virulence factors were present in all patients, with a very strong response against **Tir** (up to a titer of 1:256,000), esp. in hemolytic-uremic syndrome patients, and lesser strong responses to the other three antigens. The titer to the antigens 60 days after hospitalization was decreased but was still highest for **Tir**. These results suggest that there is a strong immune response to **Tir**, and to a lesser extent to the other three virulence factors, following EHEC disease, indicating that these bacterial mols. are potential vaccine candidates for preventing EHEC disease. They also suggest that bacterial virulence factors that are inserted into host cells during infection by type III secretion systems (**Tir** or EspB) are still recognized by the host immune response.

REFERENCE COUNT: 2

REFERENCE(S): (1) Abe, A; J Exp Med 1998, V188, P1907 CAPLUS
(2) Bitzan, M; J Clin Microbiol 1992, V30, P1174 MEDLINE

L23 ANSWER 2 OF 16 MEDLINE
ACCESSION NUMBER: 2000316068 MEDLINE
DOCUMENT NUMBER: 20316068 PubMed ID: 10858257
TITLE: Mechanical fractionation reveals structural requirements for enteropathogenic Escherichia coli **Tir** insertion into host membranes.
AUTHOR: Gauthier A; de Grado M; **Finlay B B**
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology and Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada.
SOURCE: INFECTION AND IMMUNITY, (2000 Jul) 68 (7) 4344-8. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000728
Last Updated on STN: 20000728
Entered Medline: 20000720

AB Enteropathogenic Escherichia coli (EPEC) inserts its receptor for intimate adherence (**Tir**) into host cell membranes by using a type III secretion system. Detergents are frequently used to fractionate infected host cells to investigate bacterial protein delivery into mammalian cells. In this study, we found that the Triton X-100-soluble membrane fraction from EPEC-infected HeLa cells was contaminated with bacterial proteins. We therefore applied a mechanical method of cell lysis and ultracentrifugation to fractionate infected HeLa cells to investigate the biology and biochemistry of **Tir** delivery and translocation. This method demonstrates that the translocation of **Tir** into the host cell membrane requires its transmembrane domains, but not tyrosine phosphorylation or binding to **Tir**'s ligand, intimin.

L23 ANSWER 3 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2
ACCESSION NUMBER: 1999:577042 CAPLUS
DOCUMENT NUMBER: 131:194270
TITLE: Methods for assaying type III secretion inhibitors
INVENTOR(S): Finlay, Brett B.; **Kenny, Brendan**; **Stein, Marcus**
PATENT ASSIGNEE(S): University of British Columbia, Can.

SOURCE: PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9945136	A1	19990910	WO 1999-CA183	19990305
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9932431	A1	19990920	AU 1999-32431	19990305

PRIORITY APPLN. INFO.: US 1998-76980 P 19980305
WO 1999-CA183 W 19990305

AB A method is provided for identifying compds. that specifically inhibit type III secretion systems that are used by several Gram-neg. animal and plant pathogens to secrete virulence factors that are crit. in causing disease. The compds. identified by this method are used as new antibacterial therapeutics. Specific inhibitors of the enteropathogenic Escherichia coli (EPEC) type III secretion system, that block EPEC signaling in host cells are identified by the use of specific mol. tools that have been developed with EPEC, including specific antibodies to secreted proteins and genetic fusions of epitope tags to genes encoding these secreted products. Promising compds. identified with the EPEC system are tested for their ability to inhibit type III secretion systems in other medically important pathogens.

REFERENCE COUNT: 2
REFERENCE(S): (1) Brett, F; WO 9740063 A 1997 CAPLUS
(2) Holden, D; WO 9617951 A 1996 CAPLUS

L23 ANSWER 4 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3
ACCESSION NUMBER: 1999:326051 CAPLUS
DOCUMENT NUMBER: 130:333761
TITLE: Pathogenic Escherichia coli intimin receptor Tir and gene tir and methods for detecting gene tir or Tir protein and for drug screening
INVENTOR(S): **Finlay, B. Brett; Kenny, Brendan; Devinney, Rebekah; Stein, Marcus**

PATENT ASSIGNEE(S): University of British Columbia, Can.
SOURCE: PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9924576	A1	19990520	WO 1998-CA1042	19981110
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9911373	A1	19990531	AU 1999-11373	19981110
EP 1029054	A1	20000823	EP 1998-954076	19981110
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: US 1997-65130 P 19971112
WO 1998-CA1042 W 19981110

AB A polypeptide, called Tir (for translocated intimin receptor), which is secreted by attaching and effacing pathogens, such as the enteropathogenic (EPEC) and enterohemorrhagic (EHEC) E. coli is disclosed. These bacterial pathogens insert their own receptors into mammalian cell surfaces, to which the bacterial pathogen then adheres to trigger addnl. host signaling events and actin nucleation. Diagnosis of disease caused by pathogenic E. coli can be performed by the use of antibodies which bind to Tir to detect the protein or the use of nucleic acid probes for detection of nucleic acids encoding Tir polypeptide. Isolated nucleic acid sequences encoding Tir polypeptide, Tir peptides, a recombinant method for producing recombinant Tir, antibodies which bind to Tir, and a kit for the detection of Tir-producing E. coli are provided. A method of immunizing a host with Tir to induce a protective immune response to Tir or a second polypeptide of interest is also provided. A method for screening for compds. which interfere with the binding of bacterial pathogens to their receptors is further provided. Thus, protein Hp90, previously believed to be a host membrane protein, has been identified as an EHEC- or EPEC-secreted protein which acts as an intimin receptor. Proteins encoded by the espA and espB genes were necessary for delivery of Tir to the host membrane.

REFERENCE COUNT: 6
REFERENCE(S):
(1) Deibel, C; Molecular Microbiology 1998, V28(3), P463 CAPLUS
(2) Kenny, B; Cell 1997, V91, P511 CAPLUS
(3) Kenny, B; Infection and Immunity 1997, V65(7), P2528 CAPLUS
(4) Paton, A; Database EMBL - EMPRO 1998
(5) Paton, A; Infection and Immunity 1998, V66(11), P5580 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 5 OF 16 TOXLIT
ACCESSION NUMBER: 1999:65666 TOXLIT
DOCUMENT NUMBER: CA-131-194270E
TITLE: Methods for assaying type III secretion inhibitors.
AUTHOR: Finlay BB; **Kenny B**; **Stein M**
SOURCE: (1999). PCT Int. Appl. PATENT NO. 9945136 09/10/1999 (University of British Columbia).
CODEN: PIXXD2.
PUB. COUNTRY: CANADA
DOCUMENT TYPE: Patent
FILE SEGMENT: CA
LANGUAGE: English
OTHER SOURCE: CA 131:194270
ENTRY MONTH: 199910
AB A method is provided for identifying compds. that specifically inhibit type III secretion systems that are used by several Gram-neg. animal and plant pathogens to secrete virulence factors that are crit. in causing disease. The compds. identified by this method are used as new antibacterial therapeutics. Specific inhibitors of the enteropathogenic Escherichia coli (EPEC) type III secretion system, that block EPEC signaling in host cells are identified by the use of specific mol. tools that have been developed with EPEC, including specific antibodies to secreted proteins and genetic fusions of epitope tags to genes encoding these secreted products. Promising compds. identified with the EPEC system are tested for their ability to inhibit type III secretion systems in other medically important pathogens.

L23 ANSWER 6 OF 16 TOXLIT
ACCESSION NUMBER: 1999:23044 TOXLIT
DOCUMENT NUMBER: CA-130-333761K
TITLE: Pathogenic Escherichia coli intimin receptor Tir and gene tir and methods for detecting gene tir or Tir protein and for drug screening.
AUTHOR: **Finlay BB**; **Kenny B**; **Devinney R**; **Stein M**
SOURCE: (1999). PCT Int. Appl. PATENT NO. 9924576 05/20/1999

(University of British Columbia).
CODEN: PIXXD2.
PUB. COUNTRY: CANADA
DOCUMENT TYPE: Patent
FILE SEGMENT: CA
LANGUAGE: English
OTHER SOURCE: CA 130:333761
ENTRY MONTH: 199906

AB A polypeptide, called Tir (for translocated intimin receptor), which is secreted by attaching and effacing pathogens, such as the enteropathogenic (EPEC) and enterohemorrhagic (EHEC) E. coli is disclosed. These bacterial pathogens insert their own receptors into mammalian cell surfaces, to which the bacterial pathogen then adheres to trigger addnl. host signaling events and actin nucleation. Diagnosis of disease caused by pathogenic E. coli can be performed by the use of antibodies which bind to Tir to detect the protein or the use of nucleic acid probes for detection of nucleic acids encoding Tir polypeptide. Isolated nucleic acid sequences encoding Tir polypeptide, Tir peptides, a recombinant method for producing recombinant Tir, antibodies which bind to Tir, and a kit for the detection of Tir-producing E. coli are provided. A method of immunizing a host with Tir to induce a protective immune response to Tir or a second polypeptide of interest is also provided. A method for screening for compds. which interfere with the binding of bacterial pathogens to their receptors is further provided. Thus, protein Hp90, previously believed to be a host membrane protein, has been identified as an EHEC- or EPEC-secreted protein which acts as an intimin receptor. Proteins encoded by the espA and espB genes were necessary for delivery of Tir to the host membrane.

L23 ANSWER 7 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4
ACCESSION NUMBER: 1999:291203 CAPLUS
DOCUMENT NUMBER: 131:85390
TITLE: Enterohemorrhagic Escherichia coli O157:H7 produces Tir, which is translocated to the host cell membrane but is not tyrosine phosphorylated
AUTHOR(S): **DeVinney, Rebekah**; **Stein, Markus**; Reinscheid, Dieter; Abe, Akio; Ruschkowski, Sharon; Finlay, B. Brett
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1ZA, Can.
SOURCE: Infect. Immun. (1999), 67(5), 2389-2398
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Intimate attachment to the host cell leading to the formation of attaching and effacing (A/E) lesions is an essential feature of

enterohemorrhagic Escherichia coli (EHEC) O157:H7 pathogenesis. In a related pathogen, enteropathogenic E. coli (EPEC), this activity is dependent upon translocation of the intimin receptor, Tir, which becomes tyrosine phosphorylated within the host cell membrane. In contrast, the accumulation of tyrosine-phosphorylated proteins beneath adherent EHEC bacteria does not occur, leading to questions about whether EHEC uses a Tir-based mechanism for adherence and A/E lesion formation. In this report, we demonstrate that EHEC produces a functional Tir that is inserted into host cell membranes, where it serves as an intimin receptor. However, unlike in EPEC, in EHEC Tir is not tyrosine phosphorylated yet plays a key role in both bacterial adherence to epithelial cells and pedestal formation. EHEC, but not EPEC, was unable to synthesize Tir in Luria-Bertani medium but was able to secrete Tir into M9 medium, suggesting that Tir synthesis and secretion may be regulated differently in these two pathogens. EHEC Tir and EPEC Tir both bind intimin and focus cytoskeletal rearrangements, indicating that tyrosine phosphorylation is not needed for pedestal formation. EHEC and EPEC intimins are functionally interchangeable, but EHEC Tir shows a much greater affinity for EHEC intimin than for EPEC intimin. These findings highlight some of the differences and similarities between EHEC and EPEC virulence mechanisms, which can be exploited to further define the mol. basis of pedestal formation.

REFERENCE COUNT: 45

REFERENCE(S):
(1) Abe, A; Infect Immun 1997, V65, P3547 CAPLUS
(4) Bieber, D; Science 1998, V280, P2114 CAPLUS
(5) Brunder, W; V Mol:Microbiol 1997, V24, P767 CAPLUS
(6) Dean-Nystrom, E; Infect Immun 1998, V66, P4560 CAPLUS
(7) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 8 OF 16 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 1998187833 EMBASE

TITLE: EPEC delivers the goods.

AUTHOR: Kaper J.B.; **Finlay B.B.**; **DeVinney R.**; **Kenny B.**; **Stein M.**

CORPORATE SOURCE: J.B. Kaper, Center for Vaccine Development, University of Maryland, School of Medicine, 685 West Baltimore St, Baltimore, MD 21201, United States. jkaper@umaryland.edu

SOURCE: Trends in Microbiology, (1998) 6/5 (169-172).
Refs: 20
ISSN: 0966-842X CODEN: TRMIEA

PUBLISHER IDENT.: S 0966-842X(98)01266-9

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Note
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
048 Gastroenterology
LANGUAGE: English

L23 ANSWER 9 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 5
ACCESSION NUMBER: 1997:717933 CAPLUS
DOCUMENT NUMBER: 128:33769
TITLE: Pathogenic Escherichia coli associated protein EspA and espA gene encoding EspA
INVENTOR(S): Finlay, B. Brett; **Stein, Markus**; **Kenny, Brendan**
PATENT ASSIGNEE(S): University of British Columbia, Can.; Finlay, B. Brett; Stein, Markus; Kenny, Brendan
SOURCE: PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9740063	A2	19971030	WO 1997-CA265	19970423
WO 9740063	A3	19980326		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2252372	AA	19971030	CA 1997-2252372	19970423
AU 9725628	A1	19971112	AU 1997-25628	19970423
EP 904288	A2	19990331	EP 1997-917185	19970423
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
CN 1222937	A	19990714	CN 1997-195690	19970423
BR 9710433	A	19991005	BR 1997-10433	19970423
JP 2000511771	T2	20000912	JP 1997-537532	19970423

PRIORITY APPLN. INFO.: US 1996-15999 P 19960423
WO 1997-CA265 W 19970423

AB The present invention provides the EspA polypeptide, which is secreted by pathogenic E. coli, such as the enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC). Diagnosis of disease caused by such pathogenic E. coli can be performed by std.

techniques, such as those based upon the use of antibodies which bind to EspA to detect the protein, as well as those based on the use of nucleic acid probes for detection of nucleic acids encoding EspA protein. The invention also provides isolated nucleic acid sequences encoding EspA, EspA polypeptide, EspA peptides, a method for producing recombinant EspA, antibodies which bind to EspA, and a kit for the detection of EspA-producing E. coli. The invention also provides a method of immunizing a host with EspA to induce a protective immune response to EspA. DNA sequence of espA gene was analyzed, plasmid encoding a mutant espA gene was constructed, abolished EspA secretion and virulence of pathogenic E. coli by disrupting espA gene were obsd., and assay of screening inhibitors of bacterial type III secretion was developed.

L23 ANSWER 10 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 6
ACCESSION NUMBER: 1997:596403 CAPLUS
DOCUMENT NUMBER: 127:290298
TITLE: Characterization of two virulence proteins secreted by rabbit enteropathogenic Escherichia coli, EspA and EspB, whose maximal expression is sensitive to host body temperature
AUTHOR(S): Abe, Akio; **Kenny, Brendan**; **Stein, Markus**; Finlay, B. Brett
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Infect. Immun. (1997), 65(9), 3547-3555
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Enteropathogenic Escherichia coli (EPEC) and rabbit EPEC (RDEC-1) cause unique histopathol. features on intestinal mucosa, including attaching/effacing (A/E) lesions. Due to the human specificity of EPEC, RDEC-1 has been used as an animal model to study EPEC pathogenesis. At least two of the previously identified EPEC-secreted proteins, EspA and EspB, are required for triggering host epithelial signal transduction pathways, intimate adherence, and A/E lesions. However, the functions of these secreted proteins and their roles in pathogenesis have not been characterized. To investigate the function of EspA and EspB in RDEC-1, the espA and espB genes were cloned and their sequences were compared to that of EPEC O127. The EspA proteins showed high similarity (88.5% identity), while EspB was heterogeneous in internal regions (69.8% identity). However, RDEC-1 EspB was identical to that of enterohemorrhagic E. coli serotype O26. Mutations in RDEC-1 espA and espB revealed that the corresponding RDEC-1 gene products are essential for triggering of host signal transduction pathways and invasion into HeLa cells. Complementation with plasmids contg. EPEC

espA or/and espB genes into RDEC-1 mutant strains demonstrated that they were functionally interchangeable, although the EPEC proteins mediated higher levels of invasion. Furthermore, maximal expression of RDEC-1 and EPEC-secreted proteins occurred at their resp. host body temps., which may contribute to the lack of EPEC infectivity in rabbits.

L23 ANSWER 11 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 7
ACCESSION NUMBER: 1997:408582 CAPLUS
DOCUMENT NUMBER: 127:119431
TITLE: Enteropathogenic Escherichia coli protein secretion is induced in response to conditions similar to those in the gastrointestinal tract
AUTHOR(S): **Kenny, Brendan**; Abe, Akio; **Stein, Markus**; Finlay, B. Brett
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T-1Z3, Can.
SOURCE: Infect. Immun. (1997), 65(7), 2606-2612
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The pathogenicity of enteropathogenic Escherichia coli (EPEC) is assocd. with the expression and secretion of specific bacterial factors. EspB is one such secreted protein which is required to trigger host signaling pathways resulting in effacement of microvilli and cytoskeletal rearrangements. These events presumably contribute to the ensuing diarrhea assocd. with EPEC infections. EPEC encounters several environmental changes and stimuli during its passage from the external environment into the host gastrointestinal tract. In this paper we show that the secretion of EspB is subject to environmental regulation, and maximal secretion occurs under conditions reminiscent of those in the gastrointestinal tract. Thus, secretion is maximal at 37.degree.C, pH 7, and physiol. osmolarity. In addn., maximal secretion requires the presence of sodium bicarbonate and calcium and is stimulated by millimolar concns. of Fe(NO3)3. The secretion of the four other EPEC-secreted proteins appears to be modulated in a manner similar to that of EspB. Our results also show that secretion is not dependent on CO2, as originally reported by Haigh et al., but that CO2 more likely acts as a component of the medium buffering system, since CO2 dependence was abolished by the use of alternative buffers.

L23 ANSWER 12 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 8
ACCESSION NUMBER: 1997:755652 CAPLUS
DOCUMENT NUMBER: 128:72707
TITLE: Enteropathogenic E. coli (EPEC) transfers its receptor for intimate adherence into mammalian

cells

AUTHOR(S): **Kenny, Brendan; DeVinney, Rebekah; Stein, Markus;** Reinscheid, Dieter J.; Frey, Elizabeth A.; **Finlay, B. Brett**

CORPORATE SOURCE: Biotechnol. Lab., Dep. Biochem. Mol. Biol., Dep. Microbiol. Immunology, Univ. British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Cell (Cambridge, Mass.) (1997), 91(4), 511-520
CODEN: CELLB5; ISSN: 0092-8674

PUBLISHER: Cell Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Enteropathogenic Escherichia coli (EPEC) belongs to a group of bacterial pathogens that induce epithelial cell actin rearrangements resulting in pedestal formation beneath adherent bacteria. This requires the secretion of specific virulence proteins needed for signal transduction and intimate adherence. EPEC interaction induces tyrosine phosphorylation of a protein in the host membrane, Hp90, which is the receptor for the EPEC outer membrane protein, intimin. Hp90-intimin interaction is essential for intimate attachment and pedestal formation. Here, we demonstrate that Hp90 is actually a bacterial protein (Tir). Thus, this bacterial pathogen inserts its own receptor into mammalian cell surfaces, to which it then adheres to trigger addnl. host signaling events and actin nucleation. It is also tyrosine-phosphorylated upon transfer into the host cell.

L23 ANSWER 13 OF 16 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1997:187776 BIOSIS

DOCUMENT NUMBER: PREV199799486979

TITLE: Enteropathogenic E. coli exploitation of host epithelial cells.

AUTHOR(S): Finlay, B. Brett (1); Ruschkowski, Sharon; **Kenny, Brendan; Stein, Markus;** Reinscheid, Dieter J.; **Stein, Murry A.;** Rosenshine, Ilan

CORPORATE SOURCE: (1) Biotechnol. Lab., Univ. B.C., Vancouver, BC V6T 1Z3 Canada

SOURCE: Ades, E. W. [Editor]; Morse, S. A. [Editor]; Rest, R. F. [Editor]. Annals of the New York Academy of Sciences, (1996) Vol. 797, pp. 26-31. Annals of the New York Academy of Sciences; Microbial pathogenesis and immune response, II.
Publisher: New York Academy of Sciences 2 East 63rd Street, New York, New York 10021, USA.
Meeting Info.: Conference New York, New York, USA October 25-28, 1995

ISSN: 0077-8923. ISBN: 1-57331-017-4 (paper), 1-57331-016-6 (cloth).
DOCUMENT TYPE: Book; Conference
LANGUAGE: English

L23 ANSWER 14 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 9
ACCESSION NUMBER: 1996:679649 CAPLUS
DOCUMENT NUMBER: 126:3760
TITLE: Characterization of EspC, a 110-kilodalton protein secreted by enteropathogenic Escherichia coli which is homologous to members of the immunoglobulin A protease-like family of secreted proteins
AUTHOR(S): **Stein, Markus**; **Kenny, Brendan**; **Stein, Murry A.**; Finlay, B. Brett
CORPORATE SOURCE: Dep. Biochem. Mol. Biol., Univ. British Columbia, Vancouver, BC, V6T-1Z3, Can.
SOURCE: J. Bacteriol. (1996), 178(22), 6546-6554
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Enteropathogenic Escherichia coli (EPEC) secretes .gtoreq.5 proteins. Two of these proteins, EspA and EspB (previously called EaeB), activate signal transduction pathways in host epithelial cells. While the role of the other three proteins (39, 40, and 110 kDa) remains undetd., secretion of all 5 proteins is under the control of perA, a known pos. regulator of several EPEC virulence factors. On the basis of N-terminal protein sequence data, we cloned and sequenced the gene which encodes the 110-kDa secreted protein and examd. its possible role in EPEC signaling and interaction with epithelial cells. In accordance with the terminol. used for espA and espB, we called this gene espC, for EPEC-secreted protein C. We found significant homol. between the predicted EspC protein sequence and a family of IgA (IgA) protease-like proteins which are widespread among pathogenic bacteria. Members of this protein family are found in avian pathogenic Escherichia coli (Tsh), Haemophilus influenzae (Hap), and Shigella flexneri (SepA). Although these proteins and EspC do not encode IgA protease activity, they have considerable homol. with IgA protease from Neisseria gonorrhoeae and H. influenzae and appear to use a export system for secretion. We found that genes homologous to espC also exist in other pathogenic bacteria which cause attaching and effacing lesions, including Hafnia alvei biotype 19982, Citrobacter freundii biotype 4280, and rabbit diarrheagenic E. coli (RDEC-1). Although these strains secrete various proteins similar in mol. size to the proteins secreted by EPEC, we did not detect secretion of a 110-kDa protein by these strains. To examine the possible role of

EspC in EPEC interactions with epithelial cells, we constructed a deletion mutant in espC by allelic exchange and characterized the mutant by std. tissue culture assays. We found that EspC is not necessary for mediating EPEC-induced signal transduction in HeLa epithelial cells and does not play a role in adherence or invasion of tissue culture cells.

L23 ANSWER 15 OF 16 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 97146492 MEDLINE
DOCUMENT NUMBER: 97146492 PubMed ID: 8993348
TITLE: Enteropathogenic E. coli exploitation of host epithelial cells.
AUTHOR: Finlay B B; Ruschkowski S; **Kenny B**; **Stein M**; Reinscheid D J; **Stein M A**; Rosenshine I
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, Canada.. bfinlay@unixg.ubc.ca
SOURCE: ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1996 Oct 25) 797 26-31. Ref: 51
Journal code: 5NM; 7506858. ISSN: 0077-8923.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970131

AB Enteropathogenic E. coli (EPEC) is a leading cause of neonatal diarrhea worldwide. These organisms adhere to the intestinal cell surface, causing rearrangement in the epithelial cell surface and underlying cytoskeleton, resulting in a structure termed an attaching/effacing (A/E) lesion. A/E lesion formation is thought necessary for EPEC-mediated disease. EPEC secretes several proteins that trigger signal transduction, intimate adherence, and cytoskeletal rearrangements in epithelial cells. Additionally, it produces intimin, an outer membrane product that mediates intimate adherence. Together these various bacterial molecules contribute to the intimate relationship that is formed by EPEC with host epithelial cells which results in A/E lesion formation and diarrhea.

L23 ANSWER 16 OF 16 MEDLINE
ACCESSION NUMBER: 93010945 MEDLINE
DOCUMENT NUMBER: 93010945 PubMed ID: 1396556
TITLE: Signal transduction between enteropathogenic Escherichia coli (EPEC) and epithelial cells: EPEC

	induces tyrosine phosphorylation of host cell proteins to initiate cytoskeletal rearrangement and bacterial uptake.
AUTHOR:	Rosenshine I; Donnenberg M S; Kaper J B; **Finlay B B**
CORPORATE SOURCE:	Department of Biochemistry, University of British Columbia, Vancouver, Canada.
SOURCE:	EMBO JOURNAL, (1992 Oct) 11 (10) 3551-60. Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY:	ENGLAND: United Kingdom Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:	English
FILE SEGMENT:	Priority Journals
ENTRY MONTH:	199211
ENTRY DATE:	Entered STN: 19930122 Last Updated on STN: 19980206 Entered Medline: 19921102

AB Upon attachment to cultured HeLa cells, enteropathogenic Escherichia coli (EPEC) induces assembly of a complex cytoskeletal structure within the eucaryotic cell, localized beneath the adherent bacterium. In addition, EPEC induces its own internalization by non-phagocytic epithelial cells. We found that after binding to the epithelial cell surface, EPEC induces tyrosine phosphorylation of three eucaryotic proteins. The major phosphorylation substrate is a **90 kDa protein** (**Hp90**). In correlation with **Hp90** tyrosine phosphorylation, the EPEC-induced cytoskeletal structure also contained tyrosine phosphorylated proteins. Using tyrosine protein kinase inhibitors and EPEC mutants (cfm) that fail to induce **Hp90** phosphorylation, we demonstrate that induction of **Hp90** phosphorylation is involved in initiation of the cytoskeletal structure assembly and in bacterial uptake. Other non-invasive EPEC mutants (eae) are still able to induce **Hp90** tyrosine phosphorylation and to initiate aggregation of the tyrosine phosphorylated proteins and some cytoskeleton components. However, eae mutants are deficient in nucleating the aggregates into an organized structure.

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transform 2 enteropathogenic strains of Escherichia coli to test for inhibitors. (51pp)

22/3,AB/12 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0219264 DBA Accession No.: 98-00861 PATENT
EspA from enteropathogenic or enterohemorrhagic Escherichia coli - vector expression in host cell for recombinant protein production for use as a recombinant vaccine
AUTHOR: Finlay B B; *Stein M"**; *Kenny B"**
CORPORATE SOURCE: Vancouver, British Columbia, Canada.
PATENT ASSIGNEE: Univ.British-Columbia 1997
PATENT NUMBER: WO 9740063 PATENT DATE: 971030 WPI ACCESSION NO.: 97-535772 (9749)
PRIORITY APPLIC. NO.: US 15999 APPLIC. DATE: 960423
NATIONAL APPLIC. NO.: WO 97CA265 APPLIC. DATE: 970423
LANGUAGE: English
ABSTRACT: A new secreted EspA protein from Escherichia coli with a mol.wt. of 25,000 by SDS-PAGE is encoded by DNA (protein and DNA sequence specified) which can be contained on a vector and used to transform a host cell for production of the recombinant protein. Also claimed is an polyclonal or monoclonal antibody which binds to the EspA protein and which can be used to detect EspA in a tissue or biological fluid sample. The presence of EspA indicates infection by enteropathic E. coli. The protein may be used to immunize a host against disease caused by EspA-producing E. coli, or ameliorating such a disease. A DNA probe that hybridizes to the espA nucleic acid molecule can be used to detect espA in a sample. Also claimed is a method of identifying a compound which inhibits bacterial type-II secretion systems, a method for producing a nonpathogenic organism, preferably E. coli, and a method of producing a fusion protein containing EspA and a target protein. (62pp)
? ds; log y

26jul00 14:55:00 User219783 Session D1611.2

PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V32, N1 (APR), P151-158
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (EPEC) induce characteristic attaching and effacing (A/E) lesions on epithelial cells. This event is mediated, in part, by binding of the bacterial outer membrane protein, *intimin"**, to a second EPEC protein, *Tir"** (*translocated"** *intimin"** receptor), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study, we have localized the *intimin"**-binding domain of *Tir"** to a central 107-amino-acid region, designated *Tir"**-M. We provide evidence that both the amino- and carboxy-termini of *Tir"** are located within the host cell. In addition, using immunogold labelling electron microscopy, we have confirmed that *intimin"** can bind independently to host cells even in the absence of *Tir"**, This *Tir"**-independent interaction and the ability of EPEC to induce A/E lesions requires an intact lectinlike module residing at the carboxy-terminus of the *intimin"** polypeptide. Using the yeast two-hybrid system and gel overlays, we show that *intimin"** can bind both *Tir"** and *Tir"**-M even when the lectin-like domain is disrupted. These data provide strong evidence that *intimin"** interacts not only with *Tir"** but also in a lectinlike manner with a host cell *intimin"** receptor.
ISSN: 0950-382X

10430360 GENUINE ARTICLE#: 182MT NUMBER OF REFERENCES: 46
TITLE: Structure of the cell-adhesion fragment of *intimin"** from *enteropathogenic"** Escherichia *coli"**
AUTHOR(S): Kelly G; Prasannan S; Daniell S; Fleming K; Frankel G; Dougan G; Connerton I; Matthews S (REPRINT)
AUTHOR(S) E-MAIL: s.j.matthews@ic.ac.uk
CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biochem, Exhibit Rd/London SW7 2AY//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AY//England/; Univ London Imperial Coll Sci Technol & Med, Ctr Struct Biol, /London SW7 2AY//England/; AFRC, Reading Lab, /Reading RG6 6BZ/Berks/England/; Univ London Imperial Coll Sci Technol & Med, Wellcome Ctr Infect DIs, /London SW7 2AY//England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: NATURE STRUCTURAL BIOLOGY, 1999, V6, N4 (APR), P313-318
PUBLISHER: NATURE AMERICA INC, 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707 USA

ISSN: 1072-8368
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (EPEC) induce gross cytoskeletal rearrangement within epithelial cells, immediately beneath the attached bacterium. The C-terminal 280 amino acid residues of *intimin"** (Int280; 30.1 kDa), a bacterial cell-adhesion molecule, mediate the intimate bacterial host-cell interaction. Recently, interest in this process has been stimulated by the discovery that the bacterial *intimin"** receptor protein (*Tir"**) is translocated into the host cell membrane, phosphorylated, and after binding *intimin"** triggers the intimate attachment. Using multidimensional nuclear magnetic resonance (NMR) and combining perdeuteration with site-specific protonation of methyl groups, we have determined the global fold of Int280. This represents one of the largest, non-oligomeric protein structures to be determined by NMR that has not been previously resolved by X-ray crystallography, Int280 comprises three domains; two immunoglobulin-like domains and a C-type lectinlike module, which define a new family of bacterial adhesion molecules. These findings also imply that carbohydrate recognition may be important in *intimin"**-mediated cell adhesion.
ISSN: 1072-8368

6/3,AB/47 (Item 17 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

10323436 GENUINE ARTICLE#: 170EB NUMBER OF REFERENCES: 33
TITLE: Phosphorylation of tyrosine 474 of the *enteropathogenic"** Escherichia *coli"** (EPEC) *Tir"** receptor molecule is essential for actin nucleating activity and is preceded by additional host modifications
AUTHOR(S): Kenny B (REPRINT)
AUTHOR(S) E-MAIL: B.Kenny@bristol.ac.uk
CORPORATE SOURCE: Univ Bristol, Dept Pathol & Microbiol, Univ Walk/Bristol BS8 1TD/Avon/England/ (REPRINT); Univ Bristol, Dept Pathol & Microbiol, /Bristol BS8 1TD/Avon/England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V31, N4 (FEB), P1229-1241
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The *enteropathogenic"** Escherichia *coli"** (EPEC) *Tir"** protein becomes tyrosine phosphorylated in host cells and displays an increase in apparent molecular mass. The interaction of *Tir"** with the EPEC outer membrane protein, *intimin"**, triggers actin nucleation beneath the adherent bacteria. The *enterohaemorrhagic"** E. *coli"** 0157:H7 (*EHEC"**) *Tir"** molecule is not tyrosine phosphorylated. In

this paper, *Tir"** tyrosine phosphorylation is shown to be essential for actin nucleation activity, but not for the increase in apparent molecular mass observed in target cells. Tyrosine phosphorylation had no role in *Tir"** molecular mass shift, indicating additional host modifications. Analysis of *Tir"** intermediates indicates that tyrosine-independent modification functions to direct *Tir"**'s correct insertion from the cytoplasm into the host membrane. Deletion analysis identified *Tir"** domains participating in translocation, association with the host membrane, modification and antibody recognition. *Intimin"** was found to bind a 55-amino-acid region (TIBA) within *Tir"** that topological and sequence analysis suggests is located in an extracellular loop. Homologous TIBA sequences exist in integrins, which also bind *intimin"**. Collectively, this study provides definitive evidence for the importance of tyrosine phosphorylation for EPEC *Tir"** function and reveals differences in the pathogenicity of EPEC and *EHEC"**, The data also suggest a mechanism for *Tir"** insertion into the host membrane, as well as providing clues to the mode of *intimin"**-integrin interaction.
ISSN: 0950-382X

6/3,AB/48 (Item 18 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

10270099 GENUINE ARTICLE#: 165AC NUMBER OF REFERENCES: 32
TITLE: Detection of shiga-like toxin (stx(1) and stx(2)), *intimin"** (*eaeA"**), and *enterohemorrhagic"** Escherichia *coli"** (*EHEC"**) hemolysin (*EHEC"** hlyA) genes in animal feces by multiplex PCR
AUTHOR(S): Fagan PK; Hornitzky MA; Bettelheim KA; Djordjevic SP (REPRINT)
AUTHOR(S) E-MAIL: steve.djordjevic@agric.nsw.gov.au
CORPORATE SOURCE: New S Wales Agr, Elizabeth Macarthur Agr Inst, Private Mail Bag 8/Camden/NSW 2570/Australia/ (REPRINT); New S Wales Agr, Elizabeth Macarthur Agr Inst, /Camden/NSW 2570/Australia/; Fairfield Hosp, Victorian Infect Dis Reference Lab, /Fairfield/Vic 3078/Australia/
PUBLICATION TYPE: JOURNAL
PUBLICATION: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 1999, V65, N2 (FEB), P 868-872
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171 USA
ISSN: 0099-2240
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: A multiplex PCR was developed for the rapid detection of genes encoding Shiga toxins 1 and 2 (stx(1) and stx(2)), *intimin"** (*eaeA"**), and enterohemolysin A (hlyA) in 444 fecal samples derived from healthy and clinically affected cattle, sheep, pigs, and goats. The method involved non-solvent-based extraction of nucleic acid from an aliquot of an overnight culture of feces in EC (modified) broth. The

detection limit of the assay for both fecal samples and pure cultures was between 18 and 37 genome equivalents. stx(1) and hlyA were the most commonly encountered virulence factors.
ISSN: 0099-2240

6/3,AB/49 (Item 19 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

10198343 GENUINE ARTICLE#: 159TW NUMBER OF REFERENCES: 28
TITLE: Virulence genes of Shiga toxin-producing Escherichia coli isolated from food, animals and humans
AUTHOR(S): Meng JH (REPRINT); Zhao SH; Doyle MP
AUTHOR(S) E-MAIL: jm332@umail.umd.edu
CORPORATE SOURCE: Univ Maryland, Dept Nutr & Food Sci, /College Pk//MD/20742 (REPRINT); Univ Maryland, Dept Nutr & Food Sci, /College Pk//MD/20742; Univ Georgia, Ctr Food Safety & Qual Enhancement, /Griffin//GA/30223; Univ Georgia, Dept Food Sci & Technol, /Griffin//GA/30223
PUBLICATION TYPE: JOURNAL
PUBLICATION: INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, 1998, V45, N3 (DEC 22), P229-235
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
ISSN: 0168-1605
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The presence of virulence genes, encoding *enterohemorrhagic"** Escherichia *coli"** (*EHEC"**)-hemolysin (*EHEC"**-hlyA), *intimin"** (eae), and Shiga toxins 1 (stx1) and 2 (stx2), in 178 isolates of pathogenic E. *coli"**, was determined using the polymerase chain reaction with primers specific for each virulence gene. The tested organisms were 120 isolates of E. call O157:H7 from human patients, cattle, sheep and foods, 16 non-O157:H7 *EHEC"** isolates from patients suffering from hemorrhagic colitis or hemolytic uremic syndrome, 15 non-O157:H7 Shiga toxin-producing E. *coli"** (STEC) isolates from cattle and foods, 26 isolates of *enteropathogenic"** E. *coli"** (EPEC), enteroinvasive E. *coli"** (EIEC) and enterotoxigenic E. *coli"** (ETEC), and an E. *coli"** K12 strain. Results revealed that all isolates of O157:H7 carried *EHEC"**-hlyA, ene, and one or both stx genes; 15 of the 16 non-O157:H7 *EHEC"** isolates had *EHEC"**-hlyA, but all possessed eae and one or both stx genes; only seven of the 15 non-O157 STEC isolated from cattle and foods contained both *EHEC"** -hlyA and eae genes. The EPEC, EIEC, ETEC, and the E. *coli"** K12 strain did not carry these virulence genes, except eight EPEC isolates were positive for eae. Results suggest that a combination of *EHEC"** -hlyA and eae genes could serve as markers to differentiate *EHEC"** from less pathogenic STEC, and other pathogenic or non-pathogenic E. *coli"**.
ISSN: 0168-1605

Searcher : Shears 308-4994

6/3,AB/50 (Item 20 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

09901899 GENUINE ARTICLE#: 125WL NUMBER OF REFERENCES: 21
TITLE: The medium is the messenger
AUTHOR(S): Phillips AD (REPRINT)
CORPORATE SOURCE: UNIV LONDON ROYAL FREE HOSP,DEPT PAEDIAT GASTROENTEROL, POND ST/LONDON NW3 2QG//ENGLAND/ (REPRINT)
PUBLICATION TYPE: JOURNAL
PUBLICATION: GUT, 1998, V43, N4 (OCT), P456-457
PUBLISHER: BRITISH MED JOURNAL PUBL GROUP, BRITISH MED ASSOC HOUSE, TAVISTOCK SQUARE, LONDON WC1H 9JR, ENGLAND
ISSN: 0017-5749
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (EPEC), like many bacterial pathogens, employ a type III secretion system to deliver effector proteins across the bacterial cell, in EPEC, four proteins are known to be exported by a type III secretion system-EspA, EspB and EspD required for subversion of host cell signal transduction pathways and a *translocated"** *intimin"** receptor (*Tir"**) protein (formerly Hp90) which is tyrosine-phosphorylated following transfer to the host cell to become a receptor for *intimin"**-mediated intimate attachment and 'attaching and effacing' (A/E) lesion formation. The structural basis Mr protein translocation has yet to be fully elucidated for ally type ill secretion system. Here, we describe a novel EspA-containing filamentous organelle that is present on the bacterial surface during the early stage of A/E lesion formation, forms a physical bridge between the bacterium and the infected eukaryotic cell surface and is required for the translocation Of EspB into infected epithelial cells.
ISSN: 0017-5749

6/3,AB/51 (Item 21 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

09806492 GENUINE ARTICLE#: 115XX NUMBER OF REFERENCES: 7
TITLE: BipA affects Ca++ fluxes and phosphorylation of the *translocated"** *intimin"** receptor (*Tir"**/Hp90) in host epithelial cells infected with *enteropathogenic"** E-*coli"**
AUTHOR(S): Farris M (REPRINT); Grant A; Jane S; Chad J; OConnor CD
CORPORATE SOURCE: UNIV SOUTHAMPTON,SCH BIOL SCI, DIV BIOCHEM & MOL BIOL/SOUTHAMPTON SO16 7PX/HANTS/ENGLAND/ (REPRINT); UNIV SOUTHAMPTON,SCH BIOL SCI, DIV CELL SCI/SOUTHAMPTON SO16 7PX/HANTS/ENGLAND/
PUBLICATION TYPE: JOURNAL

PUBLICATION: BIOCHEMICAL SOCIETY TRANSACTIONS, 1998, V26, N3 (AUG), P S225-S225
PUBLISHER: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON W1N 3AJ, ENGLAND
ISSN: 0300-5127
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ISSN: 0300-5127

6/3,AB/52 (Item 22 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

09417845 GENUINE ARTICLE#: ZJ871 NUMBER OF REFERENCES: 44
TITLE: A novel EspA-associated surface organelle of *enteropathogenic"** Escherichia *coli"** involved in protein translocation into epithelial cells
AUTHOR(S): Knutton S (REPRINT); Rosenshine I; Pallen MJ; Nisan I; Neves BC; Bain C; Wolff C; Dougan G; Frankel G
CORPORATE SOURCE: UNIV BIRMINGHAM,INST CHILD HLTH/BIRMINGHAM B16 8ET/W MIDLANDS/ENGLAND/ (REPRINT); UNIV LONDON IMPERIAL COLL SCI TECHNOL & MED,DEPT BIOCHEM/LONDON SW7 2AZ//ENGLAND/; HEBREW UNIV JERUSALEM,FAC MED, DEPT MOL GENET & BIOTECHNOL/IL-91120 JERUSALEM//ISRAEL/; HEBREW UNIV JERUSALEM,FAC MED, DEPT CLIN MICROBIOL/IL-91120 JERUSALEM//ISRAEL/
PUBLICATION TYPE: JOURNAL
PUBLICATION: EMBO JOURNAL, 1998, V17, N8 (APR 15), P2166-2176
PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD, ENGLAND OX2 6DP
ISSN: 0261-4189
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (EPEC), like among bacterial pathogens, employ a type WI secretion system to deliver effector proteins across the bacterial cell, In EPEC, four proteins are known to be exported by a type III secretion system-EspA, EspB and EspD required for subversion of host sell signal transduction pathways and a *translocated"** *intimin"** receptor (*Tir"**) protein (formerly Hp90) which is tyrosine-phosphorylated following transfer to the host cell to become a receptor for *intimin"**-mediated intimate attachment and 'attaching and effacing' (A/E) lesion formation, The structural basis for protein translocation has yet to Pre fully elucidated for any type III secretion system. Here, we describe a novel EspA-confirming filamentous organelle that is present on the bacterial surface during the early stage of A/E lesion formation, forms a physical bridge between the bacterium and the infected eukaryotic cell surface and is required for the translocation of EspB into infected epithelial cells.
ISSN: 0261-4189

6/3,AB/53 (Item 23 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08996050 GENUINE ARTICLE#: YG492 NUMBER OF REFERENCES: 31
TITLE: *Enteropathogenic"** E-*coli"** (EPEC) transfers its receptor for intimate adherence into mammalian cells
AUTHOR(S): Kenny B; DeVinney R; Stein M; Reinscheid DJ; Frey EA; Finlay BB (REPRINT)
CORPORATE SOURCE: UNIV BRITISH COLUMBIA,DEPT BIOCHEM & MOL BIOL, DEPT MICROBIOL & IMMUNOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV BRITISH COLUMBIA,DEPT BIOCHEM & MOL BIOL, DEPT MICROBIOL & IMMUNOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/
PUBLICATION TYPE: JOURNAL
PUBLICATION: CELL, 1997, V91, N4 (NOV 14), P511-520
PUBLISHER: CELL PRESS, 1050 MASSACHUSETTES AVE, CIRCULATION DEPT, CAMBRIDGE, MA 02138
ISSN: 0092-8674
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** E. *coli"** (EPEC) belongs to a group of bacterial pathogens that induce epithelial cell actin rearrangements resulting in pedestal formation beneath adherent bacteria. This requires the secretion of specific virulence proteins needed for signal transduction and intimate adherence. EPEC interaction induces tyrosine phosphorylation of a protein in the host membrane, Hp90, which is the receptor for the EPEC outer membrane protein, *intimin"**. Hp90-*intimin"** interaction is essential for intimate attachment and pedestal formation. Here, we demonstrate that Hp90 is actually a bacterial protein (*Tir"**). Thus, this bacterial pathogen inserts its own receptor into mammalian cell surfaces, to which it then adheres to trigger additional host signaling events and actin nucleation. It is also tyrosine-phosphorylated upon transfer into the host cell.
ISSN: 0092-8674

6/3,AB/54 (Item 24 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08878118 GENUINE ARTICLE#: BJ63E NUMBER OF REFERENCES: 0
TITLE: Host exposure to *intimin"** (*EaeA"**) prior to infection with an attaching/effacing rabbit enteropathogen - Is *intimin"** a protective antigen?
AUTHOR(S): Agin TS (REPRINT); Noel JM; McQueen CE; Boedeker EC; Wolf MK; Keusch GT; Kawakami M
CORPORATE SOURCE: WALTER REED ARMY MED CTR,WALTER REED ARMY INST RES/WASHINGTON//DC/20307 (REPRINT)
PUBLICATION TYPE: BOOK
PUBLICATION: CYTOKINES, CHOLERA, AND THE GUT, 1995, P315-320
PUBLISHER: I O S PRESS, VAN DIEMENSTRAAT 94, 1013 CN AMSTERDAM, NETHERLANDS
ISBN: 90-5199-298-X LIBRARY OF CONGRESS ID: 96-78112
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Bacteria require the *intimin"** (*EaeA"**) protein to form attaching and effacing (A/E) lesions, characteristic of EPEC and *EHEC"** infections in humans and RDEC-1 infections in rabbits, on intestinal epithelia. We retrospectively analyzed rabbit sera for the presence of anti-*intimin"** IgG prior to infection with RDEC-1 or a derivative of RDEC-1 that expresses SLT-1, to determine if there was a correlation between prior exposure to *intimin"** and protection from disease. Five rabbits, three challenged with RDEC-1 and two challenged with RDEC-H19A, had pre-existing anti-*intimin"** IgG prior to challenge and all five animals showed good weight gain post challenge. Thirty-two rabbits lacked pre-existing anti-*intimin"** Ige and showed varing weight gain post challenge. These results suggest that the rabbits with prior exposure to *intimin"** were unlikely to be in the group exhibiting symptoms of RDEC-1 infection.

6/3,AB/55 (Item 25 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

07356412 GENUINE ARTICLE#: UJ557 NUMBER OF REFERENCES: 51
TITLE: ESPA, A PROTEIN SECRETED BY *ENTEROPATHOGENIC"** ESCHERICHIA *COLI"**, IS REQUIRED TO INDUCE SIGNALS IN EPITHELIAL CELLS
AUTHOR(S): KENNY B; LAI LC; FINLAY BB; DONNENBERG MS (Reprint)
CORPORATE SOURCE: UNIV MARYLAND,SCH MED,DIV INFECT DIS,10 S PINE ST,MSTF-900/BALTIMORE//MD/21201 (Reprint); UNIV MARYLAND,SCH MED,DIV INFECT DIS/BALTIMORE//MD/21201; UNIV BRITISH COLUMBIA,DEPT BIOCHEM & MOLEC BIOL,BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/; UNIV BRITISH COLUMBIA,DEPT MICROBIOL & IMMUNOL/VANCOUVER/BC V6T 1Z3/CANADA/
PUBLICATION: MOLECULAR MICROBIOLOGY, 1996, V20, N2 (APR), P313-323
ISSN: 0950-382X
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (EPEC) is a leading cause of infant diarrhoea. EPEC mediates several effects on host epithelial cells, including activation of signal-transduction pathways, cytoskeletal rearrangement along with pedestal and attaching/effacing lesion formation. It has been previously shown that the EPEC eaeB (espB) gene encodes a secreted protein required for signal transduction and adherence, while *eaeA"** encodes *intimin"**, an EPEC membrane protein that mediates intimate adherence and contributes to focusing of cytoskeletal proteins beneath bacteria. DNA-sequence analysis of a region between *eaeA"** and eaeB identified a predicted open reading frame (espA) that matched the amino-terminal sequence of a 25 kDa EPEC secreted protein. A mutant with a non-polar insertion in espA does not secrete this protein, activate epithelial cell signal transduction or cause cytoskeletal rearrangement. These phenotypes were complemented by a cloned espA gene. The espA mutant is also defective for invasion, It is concluded that espA encodes an EPEC secreted protein that is necessary for activating epithelial signal transduction, intimate

contact, and formation of attaching and effacing lesions, processes which are central to pathogenesis.
ISSN: 0950-382X

6/3,AB/56 (Item 26 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

06508323 GENUINE ARTICLE#: RE529 NUMBER OF REFERENCES: 27
TITLE: IDENTIFICATION OF *EAEA"** PROTEIN IN THE OUTER MEMBRANE OF ATTACHING AND EFFACING ESCHERICHIA COLI O45 FROM PIGS
AUTHOR(S): ZHU CR; HAREL J; DUMAS F; FAIRBROTHER JM (Reprint)
CORPORATE SOURCE: UNIV MONTREAL,FAC VET MED,RECH MALAD INFECT PORC GRP,CP 5000/ST HYACINTHE/PQ J2S 7C6/CANADA/ (Reprint); UNIV MONTREAL,FAC VET MED,RECH MALAD INFECT PORC GRP/ST HYACINTHE/PQ J2S 7C6/CANADA/; NATL RES COUNCIL CANADA,BIOTECHNOL RES INST/MONTREAL/PQ H4P 2R2/CANADA/
PUBLICATION: FEMS MICROBIOLOGY LETTERS, 1995, V129, N2-3 (JUN 15), P237-242
ISSN: 0378-1097
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: We have previously reported that the production of attaching and effacing lesions by Escherichia *coli"** 045 isolates from pigs is associated with the *eaeA"** (E. *coli"** attaching and effacing) gene. In the present study, expression of the *EaeA"** protein, the *eaeA"** gene product, among swine 045 E. *coli"** isolates was examined. The majority (20/22) of attaching and effacing positive, *eaeA"**(+) E. *coli"** 045 isolates, but none of ten attaching and effacing negative, *eaeA"**(-) or *eaeA"**(+) isolates, expressed a 97-kDa outer membrane protein as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (*SDS"**-*PAGE"**) and Western blot analysis. Amino-terminal amino acid sequencing demonstrated a high homology between this 97-kDa protein of swine E. *coli"** 045 and the *EaeA"** protein (*intimin"**) of human *enteropathogenic"** E. *coli"** and *enterohemorrhagic"** E. *coli"**. In addition, a serological relationship between the *EaeA"** proteins of swine 045, rabbit (RDEC-1) and human (E2348/69) attaching and effacing E. *coli"** strains was observed. Our results indicate an association between expression of the *EaeA"** protein and attaching and efficacing activity among 045 E. *coli"** isolates. The data also suggest an antigenic relatedness of the *EaeA"** proteins of swine, rabbit, and human attaching and effacing E. *coli"**.
ISSN: 0378-1097

6/3,AB/57 (Item 27 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

04739646 GENUINE ARTICLE#: LP331 NUMBER OF REFERENCES: 45

TITLE: A 2ND CHROMOSOMAL GENE NECESSARY FOR INTIMATE ATTACHMENT OF *ENTEROPATHOGENIC"** ESCHERICHIA-*COLI"** TO EPITHELIAL CELLS

AUTHOR(S): DONNENBERG MS; YU J; KAPER JB
CORPORATE SOURCE: DEPT VET AFFAIRS MED CTR,MED SERV/BALTIMORE//MD/21201 (Reprint); UNIV MARYLAND,SCH MED,DEPT MED,CTR VACCINE DEV/BALTIMORE//MD/21201; UNIV MARYLAND,SCH MED,DIV INFECT DIS/BALTIMORE//MD/21201

PUBLICATION: JOURNAL OF BACTERIOLOGY, 1993, V175, N15 (AUG), P4670-4680
ISSN: 0021-9193
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (EPEC) is capable of attaching intimately to epithelial cells and effacing their microvilli. A chromosomal locus, *eaeA"** (originally eae), is required for the intimate attachment aspect of this effect. We report the mapping of a region of the EPEC chromosome that is located immediately downstream of the *eaeA"** gene and that is also necessary for intimate attachment. An isogenic in-frame deletion mutation in one of the open reading frames identified in this region was engineered. Because the resulting mutant, like an *eaeA"** deletion mutant, is deficient in the ability to attach intimately to epithelial cells, the mutated gene is designated eaeB. Full activity is restored to the eaeB mutant when the cloned gene is reintroduced on a plasmid. The eaeB mutant remains capable of producing *intimin"**, the product of the *eaeA"** gene. No differences in the fractionation properties or electrophoretic mobility of *intimin"** are apparent in the eaeB mutant. The product of the eaeB locus was identified by in vitro transcription-translation. The nucleotide sequence of the eaeB gene predicts a protein that contains a sequence motif common to several aminotransferase enzymes. These results indicate that the attaching and effacing effect is a complex phenotype dependent on a gene cluster present on the EPEC chromosome.

ISSN: 0021-9193

6/3,AB/58 (Item 1 from file: 348)
DIALOG(R)File 348:European Patents

00954338
ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
Non-pathogenic E.Coli mutant strain, process or their preparation and uses
Nicht pathogener mutanter E. coli Stamm, Verfahren zur dessen Herstellung und Verwendung
Souches mutantes non pathogenes d'E.coli, leur procede d'obtention et leurs utilisations
PATENT ASSIGNEE:
INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE (INRA), (222991), 147, rue de l'Universite, F-75341 Paris Cedex 07, (FR), (applicant designated states: BE;DE;ES;FR;IT)
Ecole Nationale Veterinaire de Toulouse (ENVT), (2445840), 23 chemin des

Capelles, 31076 Toulouse Cedex, (FR), (applicant designated states: BE;DE;ES;FR;IT)
INVENTOR:
Milon, Alain, 13 avenue du 11 Novembre 1918, 31700 Blagnac, (FR)
De Rycke, Jean, 12 chemin de Larriou, 31820 Pibrac, (FR)
LEGAL REPRESENTATIVE:
Vialle-Presles, Marie Jose et al (75731), Cabinet Ores, 6, Avenue de Messine, 75008 Paris, (FR)
PATENT (CC, No, Kind, Date): EP 864644 A1 980916 (Basic)
APPLICATION (CC, No, Date): EP 98400093 980120;
PRIORITY (CC, No, Date): FR 97532 970120
DESIGNATED STATES: BE; DE; ES; FR; IT
INTERNATIONAL PATENT CLASS: C12N-001/20

ABSTRACT EP 864644 A1 (Translated)
Non-pathogenic Escherichia coli mutants lacking cytopathic phenotype
Non-pathogenic E. coli mutants are produced from a pathogenic strain (cytopathic for HeLa cells and able: (a) to induce formation of actin filaments right through the cells, and (b) to increase the level of vinculin) by mutagenesis and selection for loss of cytopathic capacity.
TRANSLATED ABSTRACT WORD COUNT: 51

ABSTRACT EP 864644 A1
L'invention concerne un procede d'obtention de souches mutantes non-pathogenes d'E. coli, a partir de souches pathogenes capables d'induire sur des cellules epitheliales HeLa un effet cytopathique se manifestant par la formation de cables d'actine polymerisee traversant de part en part lesdites cellules et par une augmentation de la quantite de vinculine.
L'invention concerne egalement les souches mutantes non-pathogenes susceptibles d'etre obtenues par ce procede, et leur utilisation vaccinale.
ABSTRACT WORD COUNT: 69

LANGUAGE (Publication,Procedural,Application): French; French; French
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(French)	9838	315
SPEC A	(French)	9838	6654
Total word count - document A			6969
Total word count - document B			0
Total word count - documents A + B			6969

6/3,AB/59 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0239735 DBA Accession No.: 1999-09836 PATENT
Searcher : Shears 308-4994

Escherichia coli recombinant *intimin"** receptor protein - useful for distinguishing between enteropathogenic and enterohemorrhagic infection and for therapy and diagnosis

AUTHOR: Finlay B B; Kenny B; Devinney R; Stein M
CORPORATE SOURCE: Vancouver, British Columbia, Canada.

PATENT ASSIGNEE: Univ.British-Columbia 1999
PATENT NUMBER: WO 9924576 PATENT DATE: 19990520 WPI ACCESSION NO.: 1999-337712 (1928)
PRIORITY APPLIC. NO.: US 65130 APPLIC. DATE: 19971112
NATIONAL APPLIC. NO.: WO 98CA1042 APPLIC. DATE: 19981110
LANGUAGE: English

ABSTRACT: A translocated Escherichia *coli"** *intimin"** receptor protein (I) that binds *intimin"** is new. Also claimed are: a DNA sequence (II) encoding (I) and its complements, fragments and variants; DNA probes specific for (II); vectors encoding (II) and host cells containing them; (I)-specific polyclonal or monoclonal antibody; recombinant production of (I); a fusion protein containing (I); a method for identifying modulators of (I); a method for differentiating between attaching and effacing pathogens by contacting them with an anti-(I) antibody and an anti-phosphotyrosine antibody; drug delivery to (I)-containing cells using a cell delivery vehicle; kits for the detection of (I) and (II); and a method for inducing a cell-mediated immune response in cattle or humans to a protein of interest by contacting a subject with an attenuated bacteria, where the bacterium lacks an EspA or EspB protein, and contains (II) in a fusion construct. The presence of (I) in a sample is indicative of *enteropathogenic"** or *enterohemorrhagic"** infection. (91pp)

Set	Items	Description
S7	110	S1 AND PATHOGEN? ?
S8	39	S7 AND (INTIMIN OR SDSPAGE OR SDS(W)PAGE)
S9	6	S8 NOT S5
S10	5	RD (unique items)

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? t 10/3,ab/1-5
>>>No matching display code(s) found in file(s): 65, 113

 10/3,AB/1     (Item 1 from file: 144)
DIALOG(R)File 144:Pascal
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  14451724   PASCAL No.: 00-0110706
  Rapid and sensitive detection of Escherichia coli O157:H7 in bovine
faeces by a multiplex PCR
  HU Y; ZHANG Q; MEITZLER J C
  Food Animal Health Research Program, Department of Veterinary Preventive
Medicine, Ohio Agricultural Research and Development Center, The Ohio State
University, Wooster, OH 44691, United States
  Journal: Journal of applied microbiology, 1999, 87 (6) 867-876
                          Searcher   :    Shears   308-4994
```

Language: English

Cattle are considered the major reservoir for Escherichia coli O157:H7, one of the newly emerged foodborne human *pathogens"** of animal origin and a leading cause of haemorrhagic colitis in humans. A sensitive test that can accurately and rapidly detect the organism in the food animal production environment is critically needed to monitor the emergence, transmission, and colonization of this *pathogen"** in the animal reservoir. In this study, a novel multiplex polymerase chain reaction (PCR) assay was developed by using 5 sets of primers that specifically amplify segments of the *eaeA"**, slt-I, slt-II, fliC, rfbE genes, which allowed simultaneous identification of serotype O157:H7 and its virulence factors in a single reaction. Analysis of 82 E. coli strains (49 O157:H7 and 33 non-O157:H7) demonstrated that this PCR system successfully distinguished serotype O157:H7 from other serotypes of E. coli and provided accurate profiling of the shiga-like toxins and the *intimin"** adhesin in individual strains. This multiplex PCR assay did not cross-react with the background bacterial flora in bovine faeces and could detect a single O157:H7 organism per gram of faeces when combined with an enrichment step. Together, these results indicate that the multiplex PCR assay can be used for specific identification and profiling of E. coli O157:H7 isolates, and may be applied to rapid and sensitive detection of E. coli O157:H7 in bovine faeces when combined with an enrichment step.

10/3,AB/2 (Item 2 from file: 144)
DIALOG(R)File 144:Pascal

13922117 PASCAL No.: 99-0103959

Prevalence and molecular typing of attaching and effacing Escherichia coli among calf populations in belgium

CHINA B; PIRSON V; MAINIL J

University of Liege, Faculty of Veterinary Medicine, Laboratory of Bacteriology, Liege, Belgium

Journal: Veterinary microbiology : (Amsterdam), 1998, 63 (2-4) 249-259

Language: English

Attaching and effacing Escherichia coli are involved in diarrhea in 2 to 8-week old calves. The virulence factors of these bacteria include: (i) the secretion of proteins (i.e. EspB) involved in microvilli effacement. (ii) the production of the *intimin"**, a 94 kDa outer membrane protein encoded by the *eaeA"** gene and involved in the intimate attachment of bacteria to epithelial cell and (iii) the production of verotoxins: VT 1 and/or VT2. We investigated the presence and the pathotype of these strains in several calf populations by colony hybridization or by genetic amplification. Using the colony hybridization method we showed first that only 5% of calves who died from diarrhea presented *EaeA"**+ E. coli strains and secondly that 19% of healthy calves showed an asymptomatic carriage. However, using

colony hybridization and genetic amplification, we identified *EaeA"**+ strains in 91% of calves living in farms with recurrent diarrhea problems. In 66% of the calves, there was a correlation between the presence of AEEC and diarrhea. At the pathotype level, most of the *EaeA"**+ isolates were negative for VT probes. In VT+ bacteria, the majority were VT1+. The number of VT positive bacteria was significantly higher in calves who died from diarrhea than in healthy or sick calves. This underlined the aggravating role of verotoxins in the disease. Moreover, only 25% of the bovine AEEC were positive with the EaeB probe. Surprisingly, the proportion of EaeB+ strains was significantly higher in healthy calves than in other populations.

10/3,AB/3 (Item 3 from file: 144)
DIALOG(R)File 144:Pascal

12757078 PASCAL No.: 96-0470749
Typing of bovine attaching and effacing Escherichia coli by multiplex in vitro amplification of virulence-associated genes
CHINA B; PIRSON V; MAINIL J
Laboratory of Bacteriology, Faculty of Veterinary Medicine, University of Liege, 4000 Liege, Belgium
Journal: Applied and environmental microbiology, 1996, 62 (9) 3462-3465
Language: English
Attaching and effacing Escherichia coli is a new causal agent of diarrhea in calves. Its major virulence factors are the *intimin"** protein, encoded by the *eaeA"** gene, and the Shiga-like toxins, encoded by sit genes. Because the sequences of these genes are available, we selected specific primers to amplify each virulence gene so as to develop a new identification test based on multiplex amplification of virulence-associated genes. Of 30 tested strains, 14 were *eaeA"** SUP + , 15 were *eaeA"** SUP + slt-I SUP + , 1 was *eaeA"** SUP + slt-I SUP + slt-II SUP + , and 1 was *eaeA"** SUP + slt-II SUP + . The method proved in our hands to be fast and specific and in perfect correlation with the hybridization method.

10/3,AB/4 (Item 1 from file: 266)
DIALOG(R)File 266:FEDRIP

00310411
IDENTIFYING NO.: 1K08DK02864-01 AGENCY CODE: CRISP
PATHOGENESIS & INTERVENTION STRATEGIES IN HEMORRHAGIC COLITIS
Searcher : Shears 308-4994

PRINCIPAL INVESTIGATOR: BLOOM, PETER D
ADDRESS: EMORY UNIVERSITY 1639 PIERCE DRIVE ATLANTA, GA 30322
PERFORMING ORG.: EMORY UNIVERSITY, ATLANTA, GEORGIA
SPONSORING ORG.: NAT INST OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES
FY : 1999

SUMMARY: Candidate and Environment: The candidate, Dr. Bloom completed his G.I. fellowshi p and is now a junior faculty member of the G.I. Division at the University of M aryland. With the support of an NRSA, he studied attenuated Shigella vaccine con structs as vectors for foreign antigen delivery in an animal model. This work pr ovided basic experience in molecular genetics. The candidate now seeks to become expert in these techniques under the supervision of Dr. James B. Kaper of the Center for Vaccine Development (CVD), the co-sponsor of this proposal and an aut hority in microbial molecular genetics. Because of his interest in hemolytic ure mic syndrome (HUS) stemming from his documentation of an outbreak of Shigella dy senteriae in Souther Africa, Dr. Bloom will focus his attention on an animal mod el of hemorrhagic colitis developed by the co- sponsor, Dr. Edgar C. Boedeker of the G.I. Division and CVD. UNDER Drs. Boedeker and Kaper, the candidate hopes t o acquire the molecular tools to investigate the pathogenesis of hemorrhagic col itis due to Shiga toxin producing E. coli (STEC) and to further develop his care er as an independent investigator in the outstanding research environment of the CVD. Research: The broad aim of this proposal is to use a new animal model of S TEC infection to understand the molecular pathogenesis of this disease. This app roach should aid in the development of therapeutic regimens to prevent and treat STEC disease. Over the past decade STEC have emerged as important *pathogens"**, ca using life threatening food-borne illness with numerous reports of hemorrhagic c olitis, often complicated by the HUS, occurring in sporadic and epidemic outbrea ks throughout the world. STEC produce potent protein toxins named Shiga toxins (Stx). In addition to Stx production, STEC share the ability to adhere intimately to intestinal epithelial cells by "attaching and effacing" (A/E) mechanisms. Th e most severe intestinal and renal manifestations of STEC infection result from toxin-mediated damage to microvascular endothelium, with tissue edema, inflammat ory infiltrates, cytokine production, and vascular thrombi. Endotoxin and pro-in flammatory cytokines up-regulate Stx mediated tissue injury in vitro, but these effects have not been studied in vivo. Furthermore, A/E adherence of bacteria to intestinal epithelial cells, which is encoded for in the genetic locus of enter ocyte effacement (LEE) may have a profound influence on the effectively delivery of toxin to the host. Specific aims of the proposal are to use an animal model of STEC infection to: 1. Examine the influence of A/E adherence on Stx toxicity by: a. producing a deletion mutation in *Tir"** (the *translocated"** *intimin"** receptor) a critical virulence gene of the LEE, in the toxin-producing strain RDEC-H19A; b . using the products of the genes of the LEE, *intimin"** and *TIR"**, to actively immun ize against STEC using a vaccine vector system developed for use in the rabbit m odel by the candidate. 2. Examine the sequential steps in the initiation and mai ntenance of inflammation, and the induction of vascular injury, by

ut8ilizing a selected group of antagonists in the in vivo model including: a. the cytokine IL -11 which has effects on the maintenance of intestinal epithelial barrier functi on as well as inhibitory effects on macrophage activation; b. intraluminal and s ystemic endotoxin antagonists: i. Neutrophil BPI (bactericidal/permeability incr easing); and ii. Limulus ENP (endotoxin neutralizing protein); c. anti IL- 8 ant ibody to study the acute inflammatory effects of this chemokine; d. platelet act ivator factor (PAF) antagonists which affect platelet aggregation and acute infl ammation.

10/3,AB/5 (Item 1 from file: 348)
DIALOG(R)File 348:European Patents

00985690
ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
Clostridium perfringens vaccine
Clostridium perfringens Impfstoff
Vaccine contre clostridium perfringens
PATENT ASSIGNEE:
Akzo Nobel N.V., (200754), Velperweg 76, 6824 BM Arnhem, (NL),
(applicant designated states:
AT;BE;CH;CY;DE;DK;ES;FI;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)
INVENTOR:
Sergers, Ruud Philip Antoon Maria, Groenling 3, 5831 MZ Boxmeer, (NL)
Waterfield, Nicolas Robin, 20 Lucerne Close, Cherry Hinton, Cambridge CB1 4YR, (GB)
Frandsen, Peer Lyng, 56 Borgmester Schneiders Vej, 2840 Holte, (DK)
Wells, Jeremy Mark, The Cottage Old House RD, Balsham, Cambridge CB1 GEF, (GB)
LEGAL REPRESENTATIVE:
Ogilvie-Emanuelson, Claudia Maria et al (80441), Patent Department Pharma N.V. Organon P.O. Box 20, 5340 BH Oss, (NL)
PATENT (CC, No, Kind, Date): EP 892054 A1 990120 (Basic)
APPLICATION (CC, No, Date): EP 98202032 980617;
PRIORITY (CC, No, Date): EP 97201888 970620
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/31; A61K-039/08; C07K-014/33; C12N-001/21;

ABSTRACT EP 892054 A1
The present invention relates to detoxified immunogenic derivatives of Clostridium perfringens (beta)-toxin or an immunogenic fragment thereof that have as a characteristic that they carry a mutation in the (beta)-toxin amino acid sequence, not found in the wild-type (beta)-toxin amino acid sequence. The invention also relates to genes encoding such (beta)-toxins, as well as to expression systems expressing such

(beta)-toxins. Moreover, the invention relates to bacterial expression systems expressing a native (beta)-toxin. Finally, the invention relates to vaccines based upon detoxified immunogenic derivatives of Clostridium perfringens (beta)-toxin, and methods for the preparation of such vaccines.
ABSTRACT WORD COUNT: 96

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	9903	583
SPEC A	(English)	9903	7428
Total word count - document A			8011
Total word count - document B			0
Total word count - documents A + B			8011

? ds; d 22/3,ab/1-12

Set	Items	Description
S11	661	AU=(FINLAY, B? OR FINLAY B?)
S12	250	AU=(KENNY, B? OR KENNY B?)
S13	24	AU=(DEVINNEY, R? OR DE VINNEY, R? OR DEVINNEY R? OR DE VINNEY R?)
S14	2922	AU=(STEIN, M? OR STEIN M?)
S15	3	S11 AND S12 AND S13 AND S14
S16	58	S11 AND (S12 OR S13 OR S14)
S17	17	S12 AND (S13 OR S14)
S18	6	S13 AND S14
S19	3776	S11 OR S12 OR S13 OR S14
S20	24	(S16 OR S19) AND S1
S21	24	(S15 OR S17 OR S18 OR S20) NOT (S5 OR S9)
S22	12	RD (unique items)

- Author(s)

? t 22/3,ab/1-12
>>>No matching display code(s) found in file(s): 65, 113

22/3,AB/1 (Item 1 from file: 65)
DIALOG(R)File 65:Inside Conferences

02302103 INSIDE CONFERENCE ITEM ID: CN024112431
Molecular mechanisms of enteropathogenic E. coli: Signal transduction, pedestal formation, intimate contact, and diarrhea
Finlay, B. B.; *Kenny, B."**; *Stein, M."**; Reinscheid, D.
CONFERENCE: Enteropathogenic Escherichia coli-International symposium
REVISTA DE MICROBIOLOGIA, 1996; VOL 27; SUPP 1 P: 95-98
(np), 1996
ISSN: 0001-3714
LANGUAGE: English DOCUMENT TYPE: Conference Papers
CONFERENCE EDITOR(S): Kaper, J. B.

CONFERENCE LOCATION: Sao Paulo, Brazil
CONFERENCE DATE: Aug 1995 (199508) (199508)

22/3,AB/2 (Item 2 from file: 65)
DIALOG(R)File 65:Inside Conferences

01741681 INSIDE CONFERENCE ITEM ID: CN017738833
Enteropathogenic E. coli Exploitation of Host Epithelial Cells
Finlay, B. B.; Ruschkowski, S.; *Kenny, B."**; *Stein, M."**
CONFERENCE: Microbial pathogenesis and immune response-Meeting; 2nd
ANNALS- NEW YORK ACADEMY OF SCIENCES, 1996; VOL 797 P: 26-31
New York Academy of Sciences, 1996
ISSN: 0077-8923 ISBN: 1573310166; 1573310174
LANGUAGE: English DOCUMENT TYPE: Conference Papers
CONFERENCE EDITOR(S): Ades, E. W.; Morse, S. A.; Rest, R. F.
CONFERENCE LOCATION: New York, NY
CONFERENCE DATE: Oct 1995 (199510) (199510)

22/3,AB/3 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

14307127 PASCAL No.: 99-0513847
Type III secretion-dependent hemolytic activity of enteropathogenic Escherichia coli
WARAWA J; *FINLAY B B"**; *KENNY B"**
Department of Pathology and Microbiology, School of Medical Sciences, Bristol, United Kingdom; Biotechnology Laboratory, Vancouver, British Columbia, V6T 1Z3, Canada
Journal: Infection and immunity, 1999, 67 (10) 5538-5540
Language: English
Enteropathogenic Escherichia coli (EPEC) was found to exhibit a type III secretion-dependent, contact-mediated, hemolytic activity requiring the EspA, EspB, and EspD secreted proteins. EspB and EspD display homology to pore-forming molecules. Our data suggest a mechanism to explain the requirement for all three Esp proteins in the transfer of EPEC proteins, such as *Tir"**, into target cells.

22/3,AB/4 (Item 2 from file: 144)
DIALOG(R)File 144:Pascal

14080585 PASCAL No.: 99-0273444

Enteropathogenic Escherichia coli : cellular harassment
Host-microbe interactions: bacteria
*DEVINNEY R"**; KNOECHEL D G; *FINLAY B B"**
COSSART Pascale, ed; MILLER Jeff F, ed
Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada
Unite des Interations Bacteries-Cellules, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, France; University of California Los Angeles School of Medicine, Dept of Microbiology and Immunology, 10833 Le Conte Ave., Los Angeles, CA 90024, United States
Journal: Current opinion in microbiology, 1999, 2 (1) 83-88
Language: English
The mechanisms by which enteropathogenic Escherichia coli (EPEC) mediates diarrhea remain a mystery. Recently a number of interesting and at times surprising results have come from studying EPEC interactions with host cells. Identification and characterization of bacterial factors, including *Tir"**, EspA, EspB and EspD, and host responses have expanded our grasp of the diverse effects of EPEC on host cells.

22/3,AB/5 (Item 3 from file: 144)
DIALOG(R)File 144:Pascal

13217033 PASCAL No.: 97-0484125
Characterization of two virulence proteins secreted by rabbit enteropathogenic Escherichia coli, EspA and EspB, whose maximal expression is sensitive to host body temperature
ABE A; *KENNY B"**; *STEIN M"**; FINLAY B B
Biotechnology, Laboratory, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada; Department of Bacteriology, The Kitasato Institute, Minato-ku, Tokyo 108, Japan
Journal: Infection and immunity, 1997, 65 (9) 3547-3555
Language: English
Enteropathogenic Escherichia coli (EPEC) and rabbit EPEC (RDEC-1) cause unique histopathological features on intestinal mucosa, including attaching/effacing (A/E) lesions. Due to the human specificity of EPEC, RDEC-I has been used as an animal model to study EPEC pathogenesis. At least two of the previously identified EPEC-secreted proteins, EspA and EspB, are required for triggering host epithelial signal transduction pathways, intimate adherence, and A/E lesions. However, the functions of these secreted proteins and their roles in pathogenesis have not been characterized. To investigate the function of EspA and EspB in RDEC-1, the espA and espB genes were cloned and their sequences were compared to that of EPEC 0127. The EspA proteins showed high similarity (88.5% identity), while EspB was heterogeneous in internal regions (69.8% identity). However, RDEC-I EspB was identical to that of enterohemorrhagic E. coli serotype

026. Mutations in RDEC-I espA and espB revealed that the corresponding RDEC-I gene products are essential for triggering of host signal transduction pathways and invasion into HeLa cells. Complementation with plasmids containing EPEC espA or/and espB genes into RDEC-I mutant strains demonstrated that they were functionally interchangeable, although the EPEC proteins mediated higher levels of invasion. Furthermore, maximal expression of RDEC-1 and EPEC-secreted proteins occurred at their respective host body temperatures, which may contribute to the lack of EPEC infectivity in rabbits.

22/3,AB/6 (Item 4 from file: 144)
DIALOG(R)File 144:Pascal

13078049 PASCAL No.: 97-0369601

Enteropathogenic Escherichia coli protein secretion is induced in response to conditions similar to those in the gastrointestinal tract

*KENNY B"**; ABE A; *STEIN M"**; FINLAY B B

Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, V6T-1Z3, Canada

Journal: Infection and immunity, 1997, 65 (7) 2606-2612

Language: English

The pathogenicity of enteropathogenic Escherichia coli (EPEC) is associated with the expression and secretion of specific bacterial factors. EspB is one such secreted protein which is required to trigger host signaling pathways resulting in effacement of microvilli and cytoskeletal rearrangements. These events presumably contribute to the ensuing diarrhea associated with EPEC infections. EPEC encounters several environmental changes and stimuli during its passage from the external environment into the host gastrointestinal tract. In this paper we show that the secretion of EspB is subject to environmental regulation, and maximal secretion occurs under conditions reminiscent of those in the gastrointestinal tract. Thus, secretion is maximal at 37 Degree C, pH 7, and physiological osmolarity. In addition, maximal secretion requires the presence of sodium bicarbonate and calcium and is stimulated by millimolar concentrations of Fe(NO SUB 3) SUB 3 . The secretion of the four other EPEC-secreted proteins appears to be modulated in a manner similar to that of EspB. Our results also show that secretion is not dependent on CO SUB z , as originally reported by Haigh et al. (FEMS Microbiol. Lett. 129: 63-67, 1995), but that CO SUB z more likely acts as a component of the medium buffering system, since CO SUB 2 dependence was abolished by the use of alternative buffers.

22/3,AB/7 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08760754 GENUINE ARTICLE#: XT420 NUMBER OF REFERENCES: 40
TITLE: Characterization of two virulence proteins secreted by rabbit enteropathogenic Escherichia coli, EspA and EspB, whose maximal expression is sensitive to host body temperature
AUTHOR(S): Abe P; *Kenny B"**; *Stein M"**; Finlay BB (REPRINT)
CORPORATE SOURCE: UNIV BRITISH COLUMBIA,BIOTECHNOL LAB, ROOM 237, WESBROOK BLDG, 6174 UNIV BLVD/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV BRITISH COLUMBIA,BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/; KITASATO INST,DEPT BACTERIOL, MINATO KU/TOKYO 108//JAPAN/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1997, V65, N9 (SEP), P3547-3555
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Enteropathogenic Escherichia coli (EPEC) and rabbit EPEC (RDEC-1) cause unique histopathological features on intestinal mucosa, including attaching/effacing (A/E) lesions. Due to the human specificity of EPEC, RDEC-1 has been used as an animal model to study EPEC pathogenesis. At least two of the previously identified FPEC-secreted proteins, EspA and EspB, are required for triggering host epithelial signal transduction pathways, intimate adherence, and A/E lesions. However, the functions of these secreted proteins and their roles in pathogenesis have not been characterized. To investigate the function of EspA and EspB in RDEC-1, the espA and espB genes were cloned and their sequences were compared to that of EPEC O127, The EspA proteins showed high similarity (88.5% identity), while EspB was heterogeneous in internal regions (69.8% identity). However, RDEC-1 EspB was identical to that of enterohemorrhagic E. coli serotype O26. Mutations in RDEC-1 espA and espB revealed that the corresponding RDEC-1 gene products are essential for triggering of host signal transduction pathways and invasion into HeLa cells. Complementation with plasmids containing FPEC espA or/and espB genes into RDEC-1 mutant strains demonstrated that they were functionally interchangeable, although the FPEC proteins mediated higher levels of invasion. Furthermore, maximal expression of RDEC-1 and EPEC-secreted proteins occurred at their respective host body temperatures, which may contribute to the lack of EPEC infectivity in rabbits.
ISSN: 0019-9567

22/3,AB/8 (Item 2 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

07901289 GENUINE ARTICLE#: VR931 NUMBER OF REFERENCES: 56
TITLE: Characterization of EspC, a 110-kilodalton protein secreted by enteropathogenic Escherichia coli which is homologous to members of the immunoglobulinA protease-like family of secreted proteins
AUTHOR(S): *Stein M"**; *Kenny B"**; *Stein MA"**; Finlay BB
CORPORATE SOURCE: UNIV BRITISH COLUMBIA,DEPT BIOCHEM & MOL BIOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV BRITISH COLUMBIA,DEPT BIOCHEM & MOL BIOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/; UNIV BRITISH COLUMBIA,DEPT MICROBIOL & IMMUNOL/VANCOUVER/BC V6T 1Z3/CANADA/
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF BACTERIOLOGY, 1996, V178, N22 (NOV), P6546-6554
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0021-9193
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Enteropathogenic Escherichia coli (EPEC) secretes at least five proteins, Two of these proteins, EspA and EspB (previously called EaeB), activate signal transduction pathways in host epithelial cells. While the role of the other three proteins (39, 40, and 110 kDa) remains undetermined, secretion of all five proteins is under the control of pcrA, a known positive regulator of several EPEC, virulence factors, On the basis of amino-terminal protein sequence data, se cloned and sequenced the gene which encodes the 110-kDasecreted protein and examined its possible role in EPEC signaling and interaction with epithelial cells, In accordance with the terminology used for cspA, and espB, H-e called this gene espC, for EPEC-secreted protein C, We found significant homology between the predicted EspC protein sequence and a family of immunoglobulin A (IgA) protease-like proteins which are widespread among pathogenic bacteria, Members of this protein family are found in avian pathogenic Escherichia coli (Tsh), Haemophilus influenzae (Hap), and Shigella flexneri (SepA). Although these proteins and EspC do not encode IgA protease activity, they have considerable homology with IgA protease fromNeisseria gonorrhoeae and H. influenzae and appear to use a export system for secretion, We found that genes homologous to espC also exist in other pathogenic bacteria which cause attaching and effacing lesions, including Hafnia alvei biotype 19982, Citrobacter freundii biotype 4280, and rabbit diarrheagenic E. coil (RDEC-1). Although these strains secrete various proteins similar in molecular size to the proteins secreted by EPEC, se did not detect secretion of a 110-kDa protein by these strains, To examine the possible role of EspC in EPEC interactions with epithelial cells, we constructed a deletion mutant in espC by allelic exchange and characterized the mutant by standard tissue culture assays, We found that EspC is hot necessary for mediating EPEC-induced signal transduction in HeLa epithelial cells and does not play a role in adherence or invasion of tissue culture cells.
ISSN: 0021-9193

22/3,AB/9 (Item 1 from file: 348)
DIALOG(R)File 348:European Patents

01088531
ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
METHODS FOR ASSAYING TYPE III SECRETION INHIBITORS
PROCEDES D'ANALYSE D'INHIBITEURS DE SECRETION DE TYPE III
PATENT ASSIGNEE:
UNIVERSITY OF BRITISH COLUMBIA, (917321), Room 331, IRC Building, 2194 Health Sciences Mall, Vancouver, British Columbia, V6T 1Z3, (CA), (Applicant designated States: all)
INVENTOR:
FINLAY, Brett, B., Biotechnology Lab. 237-6174 University Boulevard, Vancouver, British Columbia V6T 1Z3, (CA)
*KENNY, Brendan"**, First floor flat 59 Manor Park Redland, Bristol BS6 7HW, (GB)
*STEIN, Marcus"**, Via Fiorentina, II, I-53100 Siena, (IT
PATENT (CC, No, Kind, Date): WO 9945136 990910
APPLICATION (CC, No, Date): WO 99937945 990305; WO 99CA183 990305
PRIORITY (CC, No, Date): US 76980 P 980305
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12Q-001/02; C12Q-001/32; C12Q-001/34; C12Q-001/42; C12Q-001/48; C12Q-001/66; G01N-033/68
LANGUAGE (Publication,Procedural,Application): English; English; English

22/3,AB/10 (Item 2 from file: 348)
DIALOG(R)File 348:European Patents

01003446
ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
PATHOGENIC ESCHERICHIA COLI ASSOCIATED PROTEIN
PATHOGENESE-PROTEIN ESPA VON ESCHERICHIA COLI
PROTEINE ASSOCIEE A UN ESCHERICHIA COLI PATHOGENE
PATENT ASSIGNEE:
THE UNIVERSITY OF BRITISH COLUMBIA, (917327), 222 Health Science Mall, I.R.C. Building, Vancouver, British Columbia V6T 1Z3, (CA), (applicant designated states:
AT;BE;CH;DE;DK;ES;FI;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)
INVENTOR:
FINLAY, B. Brett, Biotechnology Laboratory, 237-6174 University Boulevard , Vancouver, British Columbia V6T 1Z3, (CA)
*STEIN, Markus, Biotechnology Laboratory"**, 237-6174 University Boulevard, Vancouver, British Columbia V6T 1Z3, (CA)
*KENNY, Brendan, Biotechnology Laboratory"**, 237-6174 University

Boulevard, Vancouver, British Columbia V6T 1Z3, (CA
LEGAL REPRESENTATIVE:
VOSSIUS & PARTNER (100311), Postfach 86 07 67, 81634 Munchen, (DE)
PATENT (CC, No, Kind, Date): EP 904288 A2 990331 (Basic)
WO 9740063 971030
APPLICATION (CC, No, Date): EP 97917185 970423; WO 97CA265
PRIORITY (CC, No, Date): US 15999 P 960423
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C07K-014/00
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English

22/3,AB/11 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0243573 DBA Accession No.: 1999-14338 PATENT
Identifying antibacterial agents that inhibit Gram-negative type-III secretion system, for treating infections -by screening for inhibition of virulence factors secreted by this system - e.g. plasmid pMS21-mediated EspB gene, herpes simplex virus tag gene transfer and expression in Escherichia coli
AUTHOR: Finlay B B; *Kenny B"**; *Stein M"**
CORPORATE SOURCE: Vancouver, British Columbia, Canada.
PATENT ASSIGNEE: Univ.British-Columbia 1999
PATENT NUMBER: WO 9945136 PATENT DATE: 19990910 WPI ACCESSION NO.: 1999-540860 (1945)
PRIORITY APPLIC. NO.: US 76980 APPLIC. DATE: 19980305
NATIONAL APPLIC. NO.: WO 99CA183 APPLIC. DATE: 19990305
LANGUAGE: English
ABSTRACT: Identification of antibacterial agents is new and involves treating bacteria that contain a polynucleotide which encodes a protein secreted by the type-III secretion system (3SS) with a test compound and detecting secretion of the protein. A reduction of secretion, relative to that in bacteria not treated with the test compound, indicates an inhibitor of 3SS. Also claimed is a kit containing in separate containers, the bacteria and a system for detecting secretion of the protein. The antibacterial agents can be used to treat infections in humans other animals and plants, e.g. where caused by enteropathogenic or enterohemorrhagic Escherichia coli, Yershi sp., Shigella sp., Pseudomonas aeruginosa, Pseudomonas syringae, Xanthomonas campestris or many others, for analyzing the functional mechanisms of 3SS and for development of more powerful or specific inhibitors. In an example, plasmid pMS21 containing a sequence encoding the N-terminal part of protein EspB and a sequence encoding a herpes simplex virus tag against which commercial antibiotics are available was used to

Devi, S.
09/189415

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- key terms

Set   Items   Description
S1   3076   90KD? OR 90(W)(KD? OR KILOD? OR KILO(W)(D OR DA OR DALTON?
           ?))
S2   4794   (TRANSLOCAT? OR TRANS(W)LOCAT?)(W)INTIMIN(W)RECEPTOR? ? OR
           TIR OR HP90 OR HP(W)90
S3   3067   (S1 OR S2) AND (PROTEIN? ? OR POLYPROTEIN? ? OR POLYPEPTID-
           E? ? OR PEPTIDE? ?)
S4   114   S3 AND (EPEC OR EHEC OR (ENTEROHEMORRH? OR ENTEROHAEMORRH?
           OR ENTEROPATHOGEN? OR ENTERO(W)(HEMORRH? OR HAEMORRH? OR PATH-
           OGEN?))(5N)COLI OR "A/E" OR ATTACH?(1W)EFFAC?)
S5   79   S4 AND INTIMIN
S6   54   S5 AND BIND?
S7   37   RD (unique items)
>>>No matching display code(s) found in file(s): 65, 113

 7/3,AB/1   (Item 1 from file: 144)
DIALOG(R)File 144:Pascal
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  15072746   PASCAL No.: 01-0232088
  Human response to Escherichia coli O157:H7 infection : Antibodies to

secreted virulence factors

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Journal: Infection and immunity, 2000, 68 (9) 5090-5095

Language: English

Vaccination has been proposed for the prevention of disease due to *enterohemorrhagic"** Escherichia *coli"** (*EHEC"**), but the immune response following human infection, including the choice of potential antigens, has not been well characterized. To study this, sera were obtained from five pediatric patients with acute diarrhea caused by E. coli O157:H70, 8, and 60 days after hospitalization. These sera were used to examine the immune response to four different *EHEC"** virulence factors: *Tir"** (*translocated"** *intimin"** *receptor"**, which is inserted into the host cell membrane), *intimin"** (bacterial outer membrane *protein"** which *binds"** to *Tir"**), EspA (secreted *protein"** which forms filamentous structures on *EHEC"** surface), and EspB (inserted into the host membrane and cytoplasm). The response to O157:H7 lipopolysaccharide was also examined. Sera were assayed against purified recombinant *proteins"** using immunoblot analysis and by enzyme-linked immunosorbent assay to determine the sera's titers to each of the antigens in all patients. We found that there was little reaction to EspA, EspB, and *intimin"** in the acute-phase sera, although there was some reactivity to *Tir"** . By day 8, titers of antibody to all four virulence factors were present in all patients, with a very strong response against *Tir"** (up to a titer of 1:256,000), especially in hemolytic-uremic syndrome patients, and lesser strong responses to the other three antigens. The titer to the antigens 60 days after hospitalization was decreased but was still highest for *Tir"** . These results suggest that there is a strong immune response to *Tir"** , and to a lesser extent to the other three virulence factors, following *EHEC"** disease, indicating that these bacterial molecules are potential vaccine candidates for preventing *EHEC"** disease. They also suggest that bacterial virulence factors that are inserted into host cells during infection by type III secretion systems (*Tir"** or EspB) are still recognized by the host immune response.

7/3,AB/2 (Item 2 from file: 144)

DIALOG(R)File 144:Pascal

15044716 PASCAL No.: 01-0202245

*Intimin"** from Shiga toxin-producing Escherichia coli and its isolated C-terminal domain exhibit different *binding"** properties for *Tir"** and a eukaryotic surface receptor

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Journal: International journal of medical microbiology, 2001, 290 (8) 683-691
Language: English
The outer membrane *protein"** *intimin"** plays a crucial role in the *attaching"** and *effacing"** process employed by different enteropathogens to colonize the epithelial surface of their hosts. In this study we have characterized the C-terminal *binding"** domain of *intimin"** from the Shiga toxin-producing Escherichia coli strain 413/89-1, that belongs to the beta -subtype of intimins. We found that a fusion of this domain to the maltose-*binding"** *protein"** *binds"** efficiently to both the *translocated"** *intimin"** *receptor"** (*Tir"**) and the surface of uninfected eukaryotic host cells. In contrast, no such *binding"** was observed with the full-length *protein"** localized on the bacterial surface. As the C-terminal domain of *intimin"** and the full-length *protein"** differ in their *binding"** activity, we suggest that the intiminbinding domain might be controlled by the N-terminal portion of the molecule to prevent unproductive interactions with molecules in the lumen of the gut.

7/3,AB/3 (Item 3 from file: 144)
DIALOG(R)File 144:Pascal

14970659 PASCAL No.: 01-0123867
Mechanical fractionation reveals structural requirements for *enteropathogenic"** Escherichia *coli"** *Tir"** insertion into host membranes
GAUTHIER A; DE GRADO M; FINLAY B B
Department of Biochemistry and Molecular Biology and Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada
Journal: Infection and immunity, 2000, 68 (7) 4344-4348
Language: English
*Enteropathogenic"** Escherichia *coli"** (*EPEC"**) inserts its receptor for intimate adherence (*Tir"**) into host cell membranes by using a type III secretion system. Detergents are frequently used to fractionate infected host cells to investigate bacterial *protein"** delivery into mammalian cells. In this study, we found that the Triton X-100-soluble membrane fraction from *EPEC"**-infected HeLa cells was contaminated with bacterial *proteins"** . We therefore applied a mechanical method of cell lysis and ultracentrifugation to fractionate infected HeLa cells to

investigate the biology and biochemistry of *Tir"** delivery and translocation. This method demonstrates that the translocation of *Tir"** into the host cell membrane requires its transmembrane domains, but not tyrosine phosphorylation or *binding"** to *Tir"**'s ligand, *intimin"**.

7/3,AB/4 (Item 4 from file: 144)
DIALOG(R)File 144:Pascal

14713466 PASCAL No.: 00-0388975
*Intimin"** from *enteropathogenic"** Escherichia *coli"** mediates remodelling of the eukaryotic cell surface
PHILLIPS A D; GIRON J; HICKS S; DOUGAN G; FRANKEL G
University Department of Paediatric Gastroenterology, Royal Free Hospital , London NW3 2QG, United Kingdom; Centro de Investigaciones Microbiol6gicas, Benemerita Universidad Autonoma de Puebla, Puebla, Mexico; Department of Biochemistry, Imperial College of Science, Technology and Medicine, London SW7 2AZ, United Kingdom
Journal: Microbiology : (Reading), 2000, 146 (p.6) 1333-1344
Language: English
Adhesion to cultured epithelial cells by *enteropathogenic"** Escherichia *coli"** (*EPEC"**) is associated with extensive rearrangement of the host cell cytoskeleton. Evidence has been presented that *EPEC"** adhesion is associated with activation of signal transduction pathways leading to production of a characteristic histopathological feature known as the *attaching"** and *effacing"** (A/E) lesion. A/E lesion formation requires *intimin"**, an *EPEC"** adhesion molecule and several *EPEC"** secreted *proteins"** (EspA, B, D and *Tir"**) involved in cell signalling and *protein"** translocation. In this study it is shown that HEp-2 cells respond during the early stages of infection with two wild-type *EPEC"** strains (B171 and E2348/69) by producing microvillus-like processes (MLP) at the site of initial bacterial adherence. *Intimin"** appears to play a key role in MLP elongation. At later stages of infection with these wild-type *EPEC"** strains, when A/E lesions have formed, the MLP were reduced in number and length to appear as at time zero, and the cell surface in the vicinity of bacterial clusters appeared unaffected. In contrast, infection with EspA- or EspB-negative, but *intimin"**-positive, *EPEC"** strains (UMD872 and UMD864, respectively) resulted in enhanced MLP proliferation and formation of 'cage-like' structures engulfing the bacteria. Inoculating HEp-2 cells with *intimin"**-coated latex spheres induced similar 'cage-like' structures. Caco-2 cells did not show *intimin"**-induced microvillus elongation in response to *EPEC"** infection, although microvillus effacement and reduction in number occurred. Similar phenomena appeared on B171 and E2348/69 infection of paediatric intestine using in vitro organ culture, i.e. elongated

microvilli were seen in association with small colonies and at the periphery of large localized colonies, along with evidence of microvillus breakdown and debris in the colony centre. These results show that *intimin"** activates signal transduction pathways involved in the remodelling of the eukaryotic cell surface, probably via *binding"** to a receptor encoded by the host cell.

7/3,AB/5 (Item 5 from file: 144)
DIALOG(R)File 144:Pascal

14660969 PASCAL No.: 00-0333697
Crystal structure of *enteropathogenic"** Escherichia *coli"** *intimin"**-receptor complex
YU LUÓ; FREY E A; PFUETZNER R A; CREAGH A L; KNOECHEL D G; HAYNES C A; FINLAY B B; STRYNADKA N C J
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Journal: Nature : (London), 2000, 405 (6790) 1073-1077
Language: English
*Intimin"** and its *translocated"** *intimin"** *receptor"** (*Tir"**) are bacterial *proteins"** that mediate adhesion between mammalian cells and *attaching"** and *effacing"** (A/E) pathogens. *Enteropathogenic"** Escherichia *coli"** (*EPEC"**) causes significant paediatric morbidity and mortality world-wide SUP 1 , A related A/E pathogen, *enterohaemorrhagic"** E. *coli"** (*EHEC"**; O157:H7) is one of the most important food-borne pathogens in North America, Europe and Japan. A unique and essential feature of A/E bacterial pathogens is the formation of actin-rich pedestals beneath the intimately adherent bacteria and localized destruction of the intestinal brush border SUP 2 . The bacterial outer membrane adhesin, *intimin"** SUP 3 , is necessary for the production of the A/E lesion and diarrhoea SUP 4 . The A/E bacteria translocate their own receptor for *intimin"**, *Tir"** SUP 5 , into the membrane of mammalian cells using the type III secretion system. The translocated *Tir"** triggers additional host signalling events and actin nucleation, which are essential for lesion formation. Here we describe the the crystal structures of an *EPEC"** *intimin"** carboxy-terminal fragment alone and in complex with the *EPEC"** *Tir"** *intimin"**-*binding"** domain, giving insight into the molecular mechanisms of adhesion of A/E pathogens.

7/3,AB/6 (Item 6 from file: 144)

14409823 PASCAL No.: 00-0065744

Human colostrum and serum contain antibodies reactive to the *intimin"**-*binding"** region of the *enteropathogenic"** Escherichia *coli"** *translocated"** *intimin"** *receptor"**

IMPERIO SANCHES M; KELLER R; HARTLAND E L; FIGUEIREDO D M M; BATCHELOR M; MARTINEZ M B; DOUGAN G; CAREIRO-SAMPAIO M M S; FRANKEL G; TRABULSI L R

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Journal: Journal of pediatric gastroenterology and nutrition, 2000, 30 (1) 73-77

Language: English

Background: In Brazil, *enteropathogenic"** Escherichia *coli"** (*EPEC"**) diarrhoea is endemic in young infants. A characteristic feature of *EPEC"** adhesion to host cells is intimate attachment leading to the formation of distinctive "*attaching"** and *effacing"**" (A/E) lesions on mammalian cells. Two genes directly involved in intimate adhesion, eae and *tir"**, encode the adhesion molecule *intimin"** and its translocated receptor *Tir"**, respectively. The *intimin"**-*binding"** domain of *Tir"** was recently mapped to the middle part of the *polypeptide"** (*Tir"**-M), and the amino (*Tir"**-N) and carboxy (*Tir"**-C) termini were found to be located within infected host cells. Recently, it was shown that colostrum samples from mothers living in Sao Paulo contain IgA-class antibodies reactive with a number of *proteins"** associated with *EPEC"** virulence. It has also been shown that patients infected with verocytotoxin-producing E. coli 0157 can produce antibodies to *Tir"**. In the current study antibody responses to the different *Tir"** domains were analyzed in sera and colostrum samples collected in an *EPEC"**-endemic area of Brazil. Methods: Recombinant *Tir"**, *Tir"**-N, *Tir"**-M, and *Tir"**-C were expressed as His-tagged *protein"** in E. coli BL21a and purified on nickel columns. Western blot analysis was used to investigate colostrum IgA- and serum IgG-class antibodies reactive with the *Tir"** fragments. Results: Anti-*Tir"** IgG antibodies were detected in the serum of children, with (63%) or without (50%) diarrhoea. Anti-*Tir"** IgA-class antibodies were detected in all the colostrum pools tested. With the use of both serum IgG- and colostrum IgA-class antibodies, an immunodominant domain of the *Tir"**-*polypeptide"**, *Tir"** M, was identified. Conclusion: The *intimin"**-*binding"** region of *Tir"** (*Tir"**-M) is the immunodominant region of the *polypeptide"** in humans. Both serum IgG-class and colostrum IgA-class antibodies reacted predominantly with the *Tir"**-M domain.

7/3,AB/7 (Item 7 from file: 144)
DIALOG(R)File 144:Pascal

14116878 PASCAL No.: 99-0312625
*Enterohemorrhagic"** Escherichia *coli"** O157:H7 produces *Tir"**, which is translocated to the host cell membrane but is not tyrosine phosphorylated
DEVINNEY R; STEIN M; REINSCHEID D; ABE A; RUSCHKOWSKI S; FINLAY B B
Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada
Journal: Infection and immunity, 1999, 67 (5) 2389-2398
Language: English
Intimate attachment to the host cell leading to the formation of *attaching"** and *effacing"** (A/E) lesions is an essential feature of *enterohemorrhagic"** Escherichia *coli"** (*EHEC"**) O157:H7 pathogenesis. In a related pathogen, *enteropathogenic"** E. *coli"** (*EPEC"**), this activity is dependent upon translocation of the *intimin"** receptor, *Tir"** , which becomes tyrosine phosphorylated within the host cell membrane. In contrast, the accumulation of tyrosine-phosphorylated *proteins"** beneath adherent *EHEC"** bacteria does not occur, leading to questions about whether *EHEC"** uses a *Tir"** -based mechanism for adherence and A/E lesion formation. In this report, we demonstrate that *EHEC"** produces a functional *Tir"** that is inserted into host cell membranes, where it serves as an *intimin"** receptor. However, unlike in *EPEC"**, in *EHEC"** *Tir"** is not tyrosine phosphorylated yet plays a key role in both bacterial adherence to epithelial cells and pedestal formation. *EHEC"**, but not *EPEC"**, was unable to synthesize *Tir"** in Luria-Bertani medium but was able to secrete *Tir"** into M9 medium, suggesting that *Tir"** synthesis and secretion may be regulated differently in these two pathogens. *EHEC"** *Tir"** and *EPEC"** *Tir"** both *bind"** *intimin"** and focus cytoskeletal rearrangements, indicating that tyrosine phosphorylation is not needed for pedestal formation. *EHEC"** and *EPEC"** intimins are functionally interchangeable, but *EHEC"** *Tir"** shows a much greater affinity for *EHEC"** *intimin"** than for *EPEC"** *intimin"** . These findings highlight some of the differences and similarities between *EHEC"** and *EPEC"** virulence mechanisms, which can be exploited to further define the molecular basis of pedestal formation.

7/3,AB/8 (Item 8 from file: 144)
DIALOG(R)File 144:Pascal

13999988 PASCAL No.: 99-0184953
*Enteropathogenic"** Escherichia *coli"** inhibits phagocytosis
GOOSNEY D L; CELLI J; KENNY B; FINLAY B B
Biotechnology Laboratory and Departments of Microbiology & Immunology and of Biochemistry & Molecular Biology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada
Journal: Infection and immunity, 1999, 67 (2) 490-495
Language: English
*Enteropathogenic"** Escherichia *coli"** (*EPEC"**) interacts with intestinal epithelial cells, activating host signaling pathways leading to cytoskeletal rearrangements and ultimately diarrhea. In this study, we demonstrate that *EPEC"** interacts with the macrophage-like cell line J774A.1 to inhibit phagocytosis by these cells. Antiphagocytic activity was also observed in cultured RAW macrophage-like cells upon *EPEC"** infection. The *EPEC"** antiphagocytic phenotype was dependent on the type III secretion pathway of *EPEC"** and its secreted *proteins"**, including EspA, EspB, and EspD. *Intimin"** and *Tir"** mutants displayed intermediate antiphagocytic activity, suggesting that intimate attachment mediated by *intimin"**-*Tir"** *binding"** may also play a role in antiphagocytosis. Tyrosine dephosphorylation of several host *proteins"** was observed following infection with secretion-competent *EPEC"** but not with secretion-deficient mutants. Dephosphorylation was detectable 120 min after infection with *EPEC"**, directly correlating with the onset of the antiphagocytic phenotype. Inhibition of *protein"** tyrosine phosphatases by pervanadate treatment increased the number of intracellular wild-type *EPEC"** organisms to levels seen with secretion-deficient mutants, suggesting that dephosphorylation events are linked to the antiphagocytic phenotype. No tyrosine phosphatase activity was detected with the *EPEC"** -secreted *proteins"**, suggesting that *EPEC"** induces antiphagocytosis via a different mechanism than Yersinia species. Taken together, the present findings demonstrate a novel function for *EPEC"**-secreted *proteins"** in triggering macrophage *protein"** tyrosine dephosphorylation and inhibition of phagocytosis.

7/3,AB/9 (Item 9 from file: 144)
DIALOG(R)File 144:Pascal

13868156 PASCAL No.: 99-0046090
*Translocated"** *intimin"** *receptors"** (*Tir"**) of shiga-toxigenic Escherichia coli isolates belonging to serogroups O26, O111, and O157 react with sera from patients with hemolytic-uremic syndrome and exhibit marked sequence heterogeneity
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Journal: Infection and immunity, 1998, 66 (11) 5580-5586

Language: English

The capacity to form *attaching"** and *effacing"** (A/E) lesions on the surfaces of enterocytes is an important virulence trait of several enteric pathogens, including *enteropathogenic"** Escherichia *coli"** (*EPEC"**) and Shiga-toxigenic E. coli (STEC). Formation of such lesions depends upon an interaction between a bacterial outer membrane *protein"** (*intimin"**) and a bacterially encoded receptor *protein"** (*Tir"**) which is exported from the bacterium and translocated into the host cell membrane. *Intimin"**, *Tir"**, and several other *proteins"** necessary for generation of A/E lesions are encoded on a chromosomal pathogenicity island termed the locus for enterocyte effacement (LEE). Reports of sequence heterogeneity and antigenic variation in the region of *intimin"** believed to be responsible for receptor *binding"** raise the possibility that the receptor itself is also heterogeneous. We have examined this by cloning and sequencing *tir"** genes from three different STEC strains belonging to serogroups 026, 0111, and 0157. The deduced amino acid sequences for the *Tir"** homologues from these strains varied markedly, exhibiting only 65.4, 80.2, and 56.7% identity, respectively, to that recently reported for *EPEC"** *Tir"**. STEC *Tir"** is also highly immunogenic in humans. Western blots of E. coli DH5 alpha expressing the various STEC *tir"** genes cloned in pBluescript (but not E. coli DH5 alpha (pBluescript)) reacted strongly with convalescent sera from patients with hemolytic-uremic syndrome (HUS) caused by known LEE-positive STEC. Moreover, no reaction was seen when the various clone lysates were probed with serum from a patient with HUS caused by a LEE-negative STEC or with serum from a healthy individual. Covariation of exposed epitopes on both *intimin"** and *Tir"** may be a means whereby STEC avoid host immune responses without compromising adhesin-receptor interaction.

7/3,AB/10 (Item 10 from file: 144)
DIALOG(R)File 144:Pascal

13078038 PASCAL No.: 97-0369590

*Intimin"**-dependent *binding"** of *enteropathogenic"** Escherichia *coli"** to host cells triggers novel signaling events, including tvrosine phosphorylation of phospholipase C- gamma 1

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Journal: Infection and immunity, 1997, 65 (7) 2528-2536

Language: English

*Enteropathogenic"** Escherichia *coli"** (*EPEC"**) interactions with HeLa epithelial cells induced the tyrosine phosphorylation of a host *protein"** of approximately 150 kDa, Hp150. Phosphorylation of this *protein"** band was dependent on the interaction of the *EPEC"** *protein"** *intimin"** with epithelial cell surfaces and was correlated with pedestal formation. Hp150 phosphorylation was specifically inhibited by the addition of cytochalasin D, an inhibitor of actin polymerization, although this appeared to be an indirect effect preventing interaction of *intimin"** with its receptor, tyrosine-phosphorylated *Hp90"**, and thus triggering Hp150 phosphorylation. This suggests the involvement of an actin-based movement of membrane-bound tyrosine-phosphorylated *Hp90"** to allow its interaction with *intimin"** . Analysis of the tyrosine-phosphorylated Hp150 *protein"** demonstrated that it is heterogeneous in composition, with phospholipase C- gamma 1 (PLC- gamma 1) being a minor component. Activation of PLC- gamma 1 by tyrosine phosphorylation leads to inositol triphosphate and Ca SUP 2 SUP + fluxes, events detected following *EPEC"** infection. *EPEC"** also induced tyrosine dephosphorylation of host *proteins"**, including a 240-kDa host *protein"** (Hp240), following *EPEC"** infection. *Protein"** dephosphorylation appears to be a signaling event which occurs independently of *intimin"**. Inhibition of host tyrosine dephosphorylation events by the addition of the tyrosine phosphatase inhibitor sodium vanadate did not prevent actin accumulation beneath the adherent bacteria. We conclude that *EPEC"** induces two sets of signaling events following infection. One set is dependent on *EPEC"** *proteins"** secreted by the type III secretion pathway (EspA and EspB) which induces *Hp90"** tyrosine phosphorylation and dephosphorylation of host phosphotyrosine *proteins"**. The second set, which is also dependent on the first signaling events, requires *intimin"** interaction with its receptor, tyrosine-phosphorylated *Hp90"** , to trigger Hp150 and PLC- gamma 1 tyrosine phosphorylation as well as pedestal formation. Inhibition of pedestal formation by tyrosine kinase inhibitors indicates an important role for tyrosine phosphorylation events during *EPEC"** subversion of host processes.

7/3,AB/11 (Item 11 from file: 144)
DIALOG(R)File 144:Pascal

12483865 PASCAL No.: 96-0147695

Expression of *attaching"**/*effacing"** activity by *enteropathogenic"** Escherichia *coli"** dependes on growth phase, temperature, and *protein"** synthesis upon contact with epithelial cells

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Journal: Infection and immunity, 1996, 64 (3) 966-973
Language: English
*Enteropathogenic"** Escherichia *coli"** (*EPEC"**) induces tyrosine phosphorylation of a *90"**-*kDa"** *protein"** (*Hp90"**) in infected epithelial cells. This in turn facilitates intimate *binding"** of *EPEC"** via the outer membrane *protein"** *intimin"**, effacement of host cell microvilli, cytoskeletal rearrangement, and bacterial uptake. This phenotype has been commonly referred to as *attaching"**/*effacing"** (A/E). The ability of *EPEC"** to induce A/E lesions was dependent on bacterial growth phase and temperature. Early-logarithmic-phase *EPEC"** grown at 37 Degree C elicits strong A/E activity within minutes after infection of HeLa epithelial cells. *EPEC"** de novo *protein"** synthesis during the first minutes of interaction with the host cell was required to elicit A/E lesions. However, once formed, bacterial viability was not needed to maintain A/E lesions. The type of growth media and partial O SUB 2 pressure level do not seem to affect the ability of *EPEC"** to cause A/E lesions. These results indicates that the A/E activity of *EPEC"** is tightly regulated by environmental and host factors.

7/3,AB/12 (Item 1 from file: 266)
DIALOG(R)File 266:FEDRIP

00289737
IDENTIFYING NO.: 5R01AI46454-02 AGENCY CODE: CRISP
HOST CELL SIGNALING BY *EHEC"** *INTIMIN"** *PROTEIN"**
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ADDRESS: UNIVERSITY OF MASSACHUSETTS 55 LAKE AVENUE NORTH WORCESTER, MA 01655
PERFORMING ORG.: UNIVERSITY OF MASSACHUSETTS MEDICAL SCH, WORCESTER, MASSACHUSETTS
SPONSORING ORG.: NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
FY : 2001
SUMMARY: *Enterohemorrhagic"** E. *coli"** (*EHEC"**) has emerged as an important agent of diarrheal disease and the leading cause of pediatric renal failure in the U.S. Intimate at tachment to host cells is an essential step during intestinal colonization by EH EC. After initial host cell attachment, the bacterium injects into the host cell a number of molecules that trigger signaling pathways and result in the disrupt ion of the eukaryotic cytoskeleton. Among the injected *proteins"** is *Tir"**, a protei n that becomes localized in the host cell membrane and acts as a receptor for th e bacterial outer membrane *protein"** *intimin"**. *Intimin"** , encoded by the eae gene, is required for the formation of a highly organized for the formation of a highly organized cytoskeletal structure containing filamentous actin directly beneath t he bound bacterium that lifts the bacterium above the plane of the host cell mem brane on a "pedestal". Deletion mutants of eae, which cannot induce the

formatio n of this pedestal, are deficient for intestinal colonization. Thus, we postulat e that *Tir"**-*intimin"** interaction is an essential early event in the development of disease caused by *EHEC"**. We have identified regions of *intimin"** and *Tir"** that inte ract with each other , and have shown that the *Tir"**-*binding"** region of *intimin"** is sufficient to induce actin condensation after pre-infection of host cells with E . coli. A detailed understanding of *Tir"**-*intimin"** *binding"**, as well as of the molec ular signals immediately downstream of this interaction, are required to gain in sight into how *EHEC"** colonizes the intestine and promotes damage. Thus, the follo wing questions will be addressed: 1. What is the topological map of *Tir"** in the e ukaryotic membrane? 2. Is. *Tir"** *binding"** by *intimin"** sufficient to trigger actin con densation on preinfected cells? Latex beads that artificially *bind"** *TIR"** will be t ested for the ability to induce actin condensation on preinfected eukaryotic cel ls. 3. How does *intimin"** and *Tir"** recognize each other? Genetic and biochemical ap proaches, including crystallographic studies, will be pursued to understand the molecular basis for this interaction. 4. Is *Tir"**-*intimin"** interaction essential to promote intestinal colonization? Point mutations in eae and *tir"** that disrupt or restore *Tir"**-*intimin"** *binding"** will be tested for their effect on colonization in an animal model for *EHEC"** infection. 5. What mammalian cell factors interact with the cytoplasmic region(s) of *Tir"**? Mammalian cell factors that directly receive from *Tir"** the biochemical signal for actin filament formation will be identified. The proposed experiments may provide novel targets for therapeutic intervention during *EHEC"** infection, as well as provide insight into the general cellular mec hanisms by which actin assembly controlled.

7/3,AB/13 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

13059879 GENUINE ARTICLE#: 472JC NUMBER OF REFERENCES: 29
TITLE: *Enteropathogenic"** E-*coli"** *Tir"** *binds"** Nck to initiate actin pedestal formation in host cells
AUTHOR(S): Gruenheid S; DeVinney R; Bladt F; Goosney D; Gelkop S; Gish GD; Pawson T; Finlay BB (REPRINT)
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CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, 6174 Univ Blvd/Vancouver/BC V6T 1G3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1G3/Canada/; Mt Sinai Hosp, Samuel Lunenfeld Res Inst, /Toronto/ON M5G 1X5/Canada/
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LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (*EPEC"**) is a bacterial pathogen that causes infantile diarrhea worldwide(1). *EPEC"** injects a bacterial *protein"**, *translocated"** *intimin"** *receptor"** (*Tir"**), into the host-cell plasma membrane where it acts as a receptor for the bacterial outer membrane *protein"**, *intimin"**(2). The interaction of *Tir"** and *intimin"** triggers a marked rearrangement of the host actin cytoskeleton into pedestals beneath adherent bacteria. On delivery into host cells, *EPEC"** *Tir"** is phosphorylated on tyrosine 474 of the intracellular carboxy-terminal domain, an event that is required for pedestal formation(3). Despite its essential role, the function of *Tir"** tyrosine phosphorylation has not yet been elucidated. Here we show that tyrosine 474 of *Tir"** directly *binds"** the host-cell adaptor *protein"** Nck, and that Nck is required for the recruitment of both neural Wiskott-Aldrich-syndrome *protein"** (N-WASP) and the actin-related *protein"** (Arp)2/3 complex to the *EPEC"** pedestal, directly linking *Tir"** to the cytoskeleton. Cells with null alleles of both mammalian Nick genes are resistant to the effects of *EPEC"** on the actin cytoskeleton. These results implicate Nick adaptors as host-cell determinants of *EPEC"** virulence.

7/3,AB/14 (Item 2 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12995334 GENUINE ARTICLE#: 464PB NUMBER OF REFERENCES: 45
TITLE: *Intimin"**-specific immune responses prevent bacterial colonization by the *attaching"**-*effacing"** pathogen Citrobacter rodentium
AUTHOR(S): Ghaem-Maghami M; Simmons CP (REPRINT); Daniell S; Pizza M; Lewis D; Frankel G; Dougan G
AUTHOR(S) E-MAIL: c.simmons@ic.ac.uk
CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Ctr Mol Microbiol & Infect, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Ctr Mol Microbiol & Infect, /London SW7 2AZ//England/; St George Hosp, Dept Infect Dis, /London SW17 0RE//England/; Chiron Vaccines Immunol Res Inst, /I-53100 Siena//Italy/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2001, V69, N9 (SEP), P5597-5605
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The formation of *attaching"** and *effacing"** (A/E) lesions on gut enterocytes is central to the pathogenesis of *enterohemorrhagic"** (*EHEC"**) Escherichia *coli"**, *enteropathogenic"** E. *coli"** (

*EPEC"**), and the rodent pathogen Citrobacter rodentium. Genes encoding A/E lesion formation map to a chromosomal pathogenicity island termed the locus of enterocyte effacement (LEE). Here we show that the LEE-encoded *proteins"** EspA, EspB, *Tir"**, and *intimin"** are the targets of long-lived humoral immune responses in C. rodentium-infected mice. Mice infected with C. rodentium developed robust acquired immunity and were resistant to reinfection with wild-type C. rodentium or a C. rodentium derivative, DBS255(pCVD438), which expressed *intimin"** derived from *EPEC"** strain E2348/69. The receptor-*binding"** domain of *intimin"** *polypeptides"** is located within the carboxy-terminal 280 amino acids (Int280). Mucosal and systemic vaccination regimens using enterotoxin-based adjuvants were employed to elicit immune responses to recombinant Int280 alpha from *EPEC"** strain E2348/69. Mice vaccinated subcutaneously with Int280 alpha, in the absence of adjuvant, were significantly more resistant to oral challenge with DBS255(pCVD438) but not with wild-type C. rodentium. This type-specific immunity could not be overcome by employing an exposed, highly conserved domain of *intimin"** (Int(388-667)) as a vaccine. These results show that anti-*intimin"** immune responses can modulate the outcome (of a C rodentium infection and support the use of *intimin"** as a component of a type-specific *EPEC"** or *EHEC"** vaccine.

7/3,AB/15 (Item 3 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12970712 GENUINE ARTICLE#: 462BY NUMBER OF REFERENCES: 41
TITLE: The *enterohaemorrhagic"** Escherichia *coli"** (serotype O157 : H7) *Tir"** molecule is not functionally interchangeable for its *enteropathogenic"** E-*coli"** (serotype O127 : H6) homologue
AUTHOR(S): Kenny B (REPRINT)
AUTHOR(S) E-MAIL: B.Kenny@bristol.ac.uk
CORPORATE SOURCE: Univ Bristol, Dept Pathol & Microbiol, Univ Walk/Bristol BS8 1TD/Avon/England/ (REPRINT); Univ Bristol, Dept Pathol & Microbiol, /Bristol BS8 1TD/Avon/England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: CELLULAR MICROBIOLOGY, 2001, V3, N8 (AUG), P499-510
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 1462-5814
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: A major virulence determinant of enteropathogenic Escherichia coil (*EPEC"**) is the *Tir"** molecule that is translocated into the plasma membrane where it orchestrates cytoskeletal rearrangements. *Tir"** undergoes several phosphorylation events within host cells, with modification on a tyrosine essential for its actin-nucleating

function. The *EHEC"** (serotype O157:H7) *Tir"** homologue is not tyrosine phosphorylated implying that it uses an alternative mechanism to nucleate actin. This is supported in this study by the demonstration that *EHEC"** *Tir"** is unable to functionally substitute for its *EPEC"** homologue. Like *EPEC"**, the *EHEC"** *Tir"** molecule is phosphorylated within host cells, with the actin-nucleating dysfunction correlated to an altered modification profile. In contrast to *EHEC"** *Tir"**, the *EPEC"** *Tir"** molecule mediated actin nucleation whether delivered into host cells by either strain. Thus, it would appear that *EHEC"** encodes specific factor(s) that facilitate the correct modification of its *Tir"** molecule within host cells. Domain-swapping experiments revealed that the N-terminal, alpha -actinin *binding"**, *Tir"** domains were functionally interchangeable, with both the actin-nucleating dysfunction and altered modification profiles linked to the *EHEC"** C-terminal *Tir"** domain. This tyrosine-independent modification process presumably confers an advantage to *EHEC"** O157:H7 and may contribute to the prevalence of this strain in *EHEC"** disease. The presented data are also consistent with *EPEC"** and *EHEC"** sharing non-phosphotyrosine phosphorylation event(s), with an important role for such modifications in *Tir"** function. An *EHEC"**-induced phosphotyrosine dephosphorylation activity is also identified.

7/3,AB/16 (Item 4 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12626083 GENUINE ARTICLE#: 422NQ NUMBER OF REFERENCES: 62
TITLE: Site-directed mutagenesis of *intimin"** alpha modulates *intimin"** -mediated tissue tropism and host specificity
AUTHOR(S): Reece S; Simmons CP; Fitzhenry RJ; Matthews S; Phillips AD; Dougan G; Frankel G (REPRINT)
AUTHOR(S) E-MAIL: g.frankel@ic.ac.uk
CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Royal Free Hosp, Ctr Paediat Gastroenterol, /London NW3 2QG//England/; Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 2001, V40, N1 (APR), P86-98
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The hallmark of *enteropathogenic"** (*EPEC"**) and *enterohaemorrhagic"** (*EHEC"**) Escherchia *coli"** adhesion to host

cells is intimate attachment leading to the formation of distinctive ' *attaching"** and *effacing"**' lesions. This event is mediated, in part, by *binding"** of the bacterial adhesion molecule *intimin"** to a second bacterial *protein"**, *Tir"**, delivered by a type III secretion system into the host cell plasma membrane. The receptor-*binding"** activity of *intimin"** is localized to the C-terminal 280 amino acids (Int280) and at least five distinct *intimin"** types (alpha, beta, gamma, delta and epsilon) have been identified thus far. In addition to *binding"** to *Tir"**, *intimin"** can also *bind"** to a component encoded by the host. The consequence of latter *intimin"**-*binding"** activity may determine tissue tropism and host specificity. In this study we selected three amino acids in *intimin"**, which are implicated in *Tir"** *binding"**, far site-directed mutagenesis. We used the yeast two-hybrid system and gel overlays to study *intimin"**-*Tir"** *protein"** interaction. In addition, the biological consequences of the mutagenesis was tested using a number of infection models (cultured epithelial cells, human intestinal explants and a mouse model). We report that while an 1237/897A substitution (positions numbered according to Int280 alpha /whole *intimin"** alpha) in *intimin"** or did not have any affect on its biological activity, a T255/914A substitution attenuated *intimin"** activity in vivo. In contrast, the mutation V252/911A affected tissue targeting in the human intestinal explant model and attenuated the biological activity of *intimin"** in the mouse model. This study provides the first clues of the molecular basis of how *intimin"** mediates tissue tropism and host specificity.

7/3,AB/17 (Item 5 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12618914 GENUINE ARTICLE#: 423CT NUMBER OF REFERENCES: 44
TITLE: Recruitment of cytoskeletal and signaling *proteins"** to *enteropathogenic"** and *enterohemorrhagic"** Escherichia *coli"** pedestals
AUTHOR(S): Goosney DL; DeVinney R; Finlay BB (REPRINT)
AUTHOR(S) E-MAIL: bfinlay@interchange.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, Room 237,Westbrook Bldg,6174 Univ Blvd/Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/; Univ British Columbia, Dept Microbiol & Immunol, /Vancouver/BC V6T 1Z3/Canada/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2001, V69, N5 (MAY), P3315-3322
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (*EPEC"**) is a human pathogen that attaches to intestinal epithelial cells and causes chronic watery diarrhea. A close relative, *enterohemorrhagic"** E. *coli"** (*EHEC"**), causes severe bloody diarrhea and hemolytic-uremic syndrome. Both pathogens insert a *protein"**, *Tir"**, into the host cell plasma membrane where it *binds"** *intimin"**, the outer membrane ligand of *EPEC"** and *EHEC"**. This interaction triggers a cascade of signaling events within the host cell and ultimately leads to the formation of an actin-rich pedestal upon which the pathogen resides. Pedestal formation is critical in mediating *EPEC"**- and *EHEC"** -induced diarrhea, get very little is known about its composition and organization. In *EPEC"**, pedestal formation requires *Tir"** tyrosine 474 phosphorylation. In *EHEC"** *Tir"** is not tyrosine phosphorylated, yet the pedestals appear similar. The composition of the *EPEC"** and *EHEC"** pedestals was analyzed by examining numerous cytoskeletal, signaling, and adapter *proteins"**, Of the 25 *proteins"** examined, only two, calpactin and CD44, were recruited to the site of bacterial attachment independently of *Tir"**, Several others, including ezrin, talin, gelsolin, and tropomyosin, were recruited to the site of *EPEC"** attachment independently of *Tir"** tyrosine 474 phosphorylation but required *Tir"** in the host membrane, The remaining *proteins"** were recruited to the pedestal in a manner dependent on *Tir"** tyrosine phosphorylation or were not recruited at all. Differences were also found between the *EPEC"** and *EHEC"** pedestals: the adapter *proteins"** Grb2 and CrKII were recruited to the *EPEC"** pedestal but were absent in the *EHEC"** pedestal. These results demonstrate that although *EPEC"** and *EHEC"** recruit similar cytoskeletal *proteins"**, there are also significant differences in pedestal composition.

7/3,AB/18 (Item 6 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12205204 GENUINE ARTICLE#: 379JP NUMBER OF REFERENCES: 31
TITLE: Interaction of the *enteropathogenic"** Escherichia *coli"** *protein"**, *translocated"** *intimin"** *receptor"** (*Tir"**), with focal adhesion *proteins"**
AUTHOR(S): Freeman NL; Zurawski DV; Chowrashi P; Ayoob JC; Huang LL; Mittal B; Sanger JM; Sanger JW (REPRINT)
AUTHOR(S) E-MAIL: sangerj@mail.med.upenn.edu
CORPORATE SOURCE: Univ Penn, Dept Cell & Dev Biol, /Philadelphia//PA/19104 (REPRINT); Univ Penn, Dept Cell & Dev Biol, /Philadelphia//PA/19104
PUBLICATION TYPE: JOURNAL
PUBLICATION: CELL MOTILITY AND THE CYTOSKELETON, 2000, V47, N4 (DEC), P 307-318

PUBLISHER: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA
ISSN: 0886-1544
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: When *enteropathogenic"** Escherichia *coli"** (*EPEC"**) attach and infect host cells, they induce a cytoskeletal rearrangement and the formation of cytoplasmic columns of actin filaments called pedestals. The attached *EPEC"** and pedestals move over the surface of the host cell in an actin-dependent reaction [Sanger et al., 1996: Cell Motil Cytoskeleton 34:279-287]. The discovery that *EPEC"** inserts the *protein"**, *translocated"** *intimin"** *receptor"** (*Tir"**), into the membrane of host cells, where it *binds"** the *EPEC"** outer membrane *protein"**, *intimin"** [Kenny et al., 1997: Cell 91:511-520], suggests *Tir"** serves two functions: tethering the bacteria to the host cell and providing a direct connection to the host's cytoskeleton. The sequence of *Tir"** predicts a *protein"** of 56.8 kD with three domains separated by two predicted trans-membrane spanning regions. A GST-fusion *protein"** of the N-terminal 233 amino acids of *Tir"** (Tir1) *binds"** to alpha-actinin, talin, and vinculin from cell extracts. GST-Tir1 also coprecipitates purified forms of alpha-actinin, talin, and vinculin while GST alone does not *bind"** these three focal adhesion *proteins"**. Biotinylated probes of these three *proteins"** also bound Tir1 cleaved from GST. Similar associations of alpha-actinin, talin, and vinculin were also detected with the C-terminus of *Tir"**, i.e., Tir3, the last 217 amino acids. Antibody staining of *EPEC"**-infected cultured cells reveals the presence of focal adhesion *proteins"** beneath the attached bacteria. Our experiments support a model in which the cytoplasmic domains of *Tir"** recruit, a number of focal adhesion *proteins"** that can *bind"** actin filaments to form pedestals. Since pedestals also contain villin, tropomyosin and myosin IT [Sanger et al., 1996: Cell Motil. Cytoskeleton 34:279-287], the pedestals appear to be a novel structure sharing properties of both focal adhesions and microvilli. Cell Motil. Cytoskeleton 47:307-318, 2000. (C) 2000 Wiley-Liss, Inc.

7/3,AB/19 (Item 7 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

11939983 GENUINE ARTICLE#: 348HD NUMBER OF REFERENCES: 14
TITLE: *Enteropathogenic"** E-*coli"** *translocated"** *intimin"** *receptor"**, *Tir"**, interacts directly with alpha-actinin
AUTHOR(S): Goosney DL; DeVinney R; Pfuetzner RA; Frey EA; Strynadka NC; Finlay BB (REPRINT)
AUTHOR(S) E-MAIL: bfinlay@interchange.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab,

/Vancouver/BC V6T 1Z3/Canada/; Univ British Columbia, Dept Microbiol & Immunol, /Vancouver/BC V6T 1Z3/Canada/; Univ British Columbia, Dept Biochem & Mol Biol, /Vancouver/BC V6T 1Z3/Canada/
PUBLICATION TYPE: JOURNAL
PUBLICATION: CURRENT BIOLOGY, 2000, V10, N12 (JUN 15), P735-738
PUBLISHER: CURRENT BIOLOGY LTD, 84 THEOBALDS RD, LONDON WC1X 8RR, ENGLAND
ISSN: 0960-9822
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (*EPEC"**) triggers a dramatic rearrangement of the host epithelial cell actin cytoskeleton to form an *attaching"** and *effacing"** lesion, or pedestal. The pathogen remains attached extracellularly to the host cell through the pedestal for the duration of the infection. At the tip of the pedestal is a bacterial *protein"**, *Tir"**, which is secreted from the bacterium into the host cell plasma membrane, where it functions as the receptor for an *EPEC"** outer membrane *protein"**, *intimin"** [1]. Delivery of *Tir"** to the host cell results in its tyrosine phosphorylation, followed by *Tir"**-*intimin"** *binding"**. *Tir"** is believed to anchor *EPEC"** firmly to the host cell, although its direct linkage to the cytoskeleton is unknown. Here, we show that *Tir"** directly *binds"** the cytoskeletal *protein"** alpha-actinin. alpha-actinin is recruited to the pedestal in a *Tir"**-dependent manner and colocalizes with *Tir"** in infected host cells. *Binding"** is mediated through the amino terminus of *Tir"**. Recruitment of alpha-actinin occurs independently of *Tir"** tyrosine phosphorylation. Recruitment of actin, VASP, and N-WASP, however, is abolished in the absence of this tyrosine phosphorylation. These results suggest that *Tir"** plays at least three roles in the host cell during infection: *binding"** *intimin"** on *EPEC"**; mediating a stable anchor with alpha-actinin through its amino terminus in a phosphotyrosine-independent manner; and recruiting additional cytoskeletal *proteins"** at the carboxyl terminus in a phosphotyrosine-dependent manner. These findings demonstrate the first known direct linkage between extracellular *EPEC"**, through the transmembrane *protein"** *Tir"**, to the host cell actin cytoskeleton via alpha-actinin.

7/3,AB/20 (Item 8 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

11919468 GENUINE ARTICLE#: 346KM NUMBER OF REFERENCES: 26
TITLE: Human response to Escherichia coli O157 : H7 infection: Antibodies to secreted virulence factors
AUTHOR(S): Li YL; Frey E; Mackenzie AMR; Finlay BB (REPRINT)
AUTHOR(S) E-MAIL: bfinlay@interchange.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, Room 237,Wesbrook

Bldg,6174 Univ Blvd/Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/; Div Microbiol, /Ottawa/ON K1Y 4E9/Canada/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2000, V68, N9 (SEP), P5090-5095
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Vaccination has been proposed for the prevention of disease due to *enterohemorrhagic"** Escherichia *coli"** (*EHEC"**), but the immune response following human infection, including the choice of potential antigens, has not been well characterized. To study this, sera were obtained from five pediatric patients with acute diarrhea caused by E. coli O157:117 0, 8, and 60 days after hospitalization. These sera were used to examine the immune response to four different *EHEC"** virulence factors: *Tir"** (*translocated"** *intimin"** *receptor"**, which is inserted into the host cell membrane), *intimin"** (bacterial outer membrane *protein"** which *binds"** to *Tir"**), EspA (secreted *protein"** which forms filamentous structures on *EHEC"** surface), and EspB (inserted into the host membrane and cytoplasm). The response to O157:H7 lipopolysaccharide was also examined. Sera were assayed against purified recombinant *proteins"** using immunoblot analysis and by enzyme-linked immunosorbent assay to determine the sera's titers to each of the antigens in all patients, We found that there was little reaction to EspA EspB, and *intimin"** in the acute-phase sera, although there was some reactivity to *Tir"**. By day 8, titers of antibody to all four virulence factors were present in all patients, with a very strong response against *Tir"** (up to a titer of 1:256,000), especially in hemolytic-uremic syndrome patients, and lesser strong responses to the other three antigens. The titer to the antigens 60 days after hospitalization was decreased but was still highest for *Tir"**. These results suggest that there is a strong immune response to *Tir"**, and to a lesser extent to the other three virulence factors, following *EHEC"** disease, indicating that these bacterial molecules are potential vaccine candidates for preventing *EHEC"** disease. They also suggest that bacterial virulence factors that are inserted into host cells during infection by type III secretion systems (*Tir"** or EspB) are still recognized by the host immune response.

7/3,AB/21 (Item 9 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

11896203 GENUINE ARTICLE#: 344RF NUMBER OF REFERENCES: 54
TITLE: *Enteropathogenic"** Escherichia *coli"** (*EPEC"**) attachment to

epithelial cells: exploiting the host cell cytoskeleton from the outside
AUTHOR(S): Celli J; Deng WY; Finlay BB (REPRINT)
AUTHOR(S) E-MAIL: bfinlay@interchange.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, Room 237,Westbrook Bldg,6174 Univ Blvd/Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/
PUBLICATION TYPE: JOURNAL
PUBLICATION: CELLULAR MICROBIOLOGY, 2000, V2, N1 (FEB), P1-9
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 1462-5814
LANGUAGE: English DOCUMENT TYPE: REVIEW
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (*EPEC"**), a leading cause of human infantile diarrhoea, is the prototype for a family of intestinal bacterial pathogens that induce *attaching"** and *effacing"** (A/E) lesions on host cells. A/E lesions are characterized by localized effacement of the brush border of enterocytes, intimate bacterial attachment and pedestal formation beneath the adherent bacteria. As a result of some recent breakthrough discoveries, *EPEC"** has now emerged as a fascinating paradigm for the study of host-pathogen interactions and cytoskeletal rearrangements that occur at the host cell membrane. *EPEC"** uses a type III secretion machinery to attach to epithelial cells, translocating its own receptor for intimate attachment, *Tir"**, into the host cell, which then *binds"** to *intimin"** on the bacterial surface. Studies of *EPEC"**-induced cytoskeletal rearrangements have begun to provide clues as to the mechanisms used by this pathogen to subvert the host cell cytoskeleton and signalling pathways. These findings have unravelled new ways by which pathogenic bacteria exploit host processes from the cell surface and have shed new light on how *EPEC"** might cause diarrhoea.

7/3,AB/22 (Item 10 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

11889829 GENUINE ARTICLE#: 341UL NUMBER OF REFERENCES: 60
TITLE: Exploitation of host cells by *enteropathogenic"** Escherichia *coli"**
AUTHOR(S): Vallance BA; Finlay BB (REPRINT)
AUTHOR(S) E-MAIL: bfinlay@interchange.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, Room 237,Wesbrook Bldg,6174 Univ Blvd/Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/
PUBLICATION TYPE: JOURNAL
PUBLICATION: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 2000, V97, N16 (AUG 1), P8799-8806

PUBLISHER: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418 USA
ISSN: 0027-8424
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Microbial pathogens have evolved many ingenious ways to infect their hosts and cause disease, including the subversion and exploitation of target host cells. One such subversive microbe is enteropathogenic Escherichia coil (*EPEC"**), A major cause of infantile diarrhea in developing countries, *EPEC"** poses a significant health threat to children worldwide, Central to *EPEC"** -mediated disease is its colonization of the intestinal epithelium. After initial adherence, *EPEC"** causes the localized effacement of microvilli and intimately attaches to the host cell surface, forming characteristic *attaching"** and *effacing"** (A/E) lesions. Considered the prototype for a family of A/E lesion-causing bacteria, recent in vitro studies of *EPEC"** have revolutionized our understanding of how these pathogens infect their hosts and cause disease, Intimate attachment requires the type Ill-mediated secretion of bacterial *proteins"**, several of which are translocated directly into the infected cell, including the bacteria's own receptor (*Tir"**). *Binding"** to this membrane-bound, pathogen-derived *protein"** permits *EPEC"** to intimately attach to mammalian cells, The translocated *EPEC"** *proteins"** also activate signaling pathways within the underlying cell, causing the reorganization of the host actin cytoskeleton and the formation of pedestal-like structures beneath the adherent bacteria, This review explores what is known about *EPEC"**'s subversion of mammalian cell functions and how this knowledge has provided novel insights into bacterial pathogenesis and microbe-host interactions, Future studies of A/E pathogens in animal models should provide further insights into how *EPEC"** exploits not only epithelial cells but other host cells, including those of the immune system, to cause diarrheal disease.

7/3,AB/23 (Item 11 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

11709866 GENUINE ARTICLE#: 322ZH NUMBER OF REFERENCES: 59
TITLE: Structural basis for recognition of the *translocated"** *intimin"** *receptor"** (*Tir"**) by *intimin"** from *enteropathogenic"** Escherichia *coli"**
AUTHOR(S): Batchelor M; Prasannan S; Daniell S; Reece S; Connerton I; Bloomberg G; Dougan G; Frankel G (REPRINT); Matthews S
AUTHOR(S) E-MAIL: s.j.matthews@ic.ac.uk
CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Univ London

Imperial Coll Sci Technol & Med, Ctr Struct Biol, /London SW7 2AZ//England/; Univ Nottingham, Div Food Sci, /Loughborough LE12 5RD/Leics/England/; Univ Bristol, Dept Biochem, /Bristol BS8 1TD/Avon/England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: EMBO JOURNAL, 2000, V19, N11 (JUN 1), P2452-2464
PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND
ISSN: 0261-4189
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Intimin"** is a bacterial adhesion molecule involved in intimate attachment of *enteropathogenic"** and *enterohaemorrhagic"** Escherichia *coli"** to mammalian host cells. *Intimin"** targets the *translocated"** *intimin"** *receptor"** (*Tir"**), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study we localized the *Tir"**-*binding"** region of *intimin"** to the C-terminal 190 amino acids (Int190), We have also determined the region's high-resolution solution structure, which comprises an immunoglobulin domain that is intimately coupled to a novel C-type lectin domain, This fragment, which is necessary and sufficient for *Tir"** interaction, defines a new super domain in *intimin"** that exhibits striking structural similarity to the integrin-*binding"** domain of the Yersinia invasin and C-type lectin families, The extracellular portion of *intimin"** comprises an articulated rod of immunoglobulin domains extending from the bacterium surface, conveying a highly accessible 'adhesive tip' to the target cell. The interpretation of NMR-titration and mutagenesis data has enabled us to identify, for the first time, the *binding"** site for *Tir"**, which is located at the extremity of the Int190 moiety.

7/3,AB/24 (Item 12 from file: 440)

11236777 GENUINE ARTICLE#: 271PR NUMBER OF REFERENCES: 21
TITLE: Human colostrum and serum contain antibodies reactive to the *intimin"**-*binding"** region of the *enteropathogenic"** Escherichia *coli"** *translocated"** *intimin"** *receptor"**
AUTHOR(S): Sanches MI; Keller R; Hartland EL; Figueiredo DMM; Batchelor M; Martinez MB; Dougan G; Careiro-Sampaio MMS; Frankel G (REPRINT); Trabulsi LR
CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Univ Sao Paulo, Dept Immunol, /Sao Paulo//Brazil/; Univ Sao Paulo, Dept Anal Clin & Toxicol, /Sao Paulo//Brazil/
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF PEDIATRIC GASTROENTEROLOGY AND NUTRITION, 2000, V30

, N1 (JAN), P73-77
PUBLISHER: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA
ISSN: 0277-2116
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Background: In Brazil, *enteropathogenic"** Escherichia *coli"** (*EPEC"**) diarrhoea is endemic in young infants. A characteristic feature of *EPEC"** adhesion to host cells is intimate attachment leading to the formation of distinctive "*attaching"** and *effacing"** " (A/E) lesions on mammalian cells. Two genes directly involved in intimate adhesion, ene and *tir"**, encode the adhesion molecule *intimin"** and its translocated receptor *Tir"**, respectively. The *intimin"**-*binding"** domain of *Tir"** was recently mapped to the middle part of the *polypeptide"** (*Tir"**-M), and the amino (*Tir"** -N) and carboxy (*Tir"**-C) termini were found to be located within infected host cells. Recently, it was shown that colostrum samples from mothers living in Sao Paulo contain IgA-class antibodies reactive with a number of *proteins"** associated with *EPEC"** virulence. It has also been shown that patients infected with verocytotoxin-producing E. coli O157 can produce antibodies to *Tir"**. In the current study antibody responses to the different *Tir"** domains were analyzed in sera and colostrum samples collected in an *EPEC"**-endemic area of Brazil.

Methods: Recombinant *Tir"**, *Tir"**-N, *Tir"**-M, and *Tir"**-C were expressed as His-tagged *protein"** in E. coli BL21a and purified on nickel columns. Western blot analysis was used to investigate colostrum IgA- and serum IgG-class antibodies reactive with the *Tir"** fragments.

Results: Anti-*Tir"** IgG antibodies were detected in the serum of children, with (63%) or without (50%) diarrhoea, Anti-*Tir"** IgA-class antibodies were detected in all the colostrum pools tested. With the use of both serum IgG- and colostrum IgA-class antibodies, an immunodominant domain of the *Tir"**-*polypeptide"**, *Tir"** M, was identified.

Conclusion: The *intimin"**-*binding"** region of *Tir"** (*Tir"** -M) is the immunodominant region of the *polypeptide"** in humans. Both serum IgG-class and colostrum IgA-class antibodies reacted predominantly with the *Tir"**-M domain. (C) 2000 Lippincott Williams & Wilkins, Inc.

7/3,AB/25 (Item 13 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

11235993 GENUINE ARTICLE#: 271RC NUMBER OF REFERENCES: 16

TITLE: Antibody response of patients infected with verocytotoxin-producing Escherichia coli to *protein"** antigens encoded on the LEE locus

AUTHOR(S): Jenkins C; Chart H (REPRINT); Smith HR; Hartland EL; Batchelor M ; Delahay RM; Dougan G; Frankel G
AUTHOR(S) E-MAIL: hchart@phls.co.uk
CORPORATE SOURCE: Cent Publ Hlth Lab, Lab Enter Pathogens, 61 Colindale Ave/London NW9 5HT//England/ (REPRINT); Cent Publ Hlth Lab, Lab Enter Pathogens, /London NW9 5HT//England/; Univ London Sch Pharm, Dept Biochem, /London SW7 2AY//England/

PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF MEDICAL MICROBIOLOGY, 2000, V49, N1 (JAN), P97-101
PUBLISHER: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA
ISSN: 0022-2615
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Sera from patients infected with verocytotoxin-producing Escherichia coli (VTEC) O157, from patients with antibodies to E. coli O157 lipopolysaccharide (LPS) and from healthy controls were examined for antibodies to *proteins"** involved in expressing the *attaching"** and *effacing"** phenotype, After SDS-PAGE, purified recombinant *intimin"**, EspA-filament structural *protein"**, translocated *protein"** EspB and three separate domains of the *translocated"** *intimin"** *receptor"** (*Tir"**) were tested for reaction with patients' sera by immunoblotting, An ELISA was also used to detect antibodies to *intimin"** in sera from E, coli O157 LPS antibody-positive individuals. Seven of nine culture-positive patients and one control patient had antibodies to EspA, Five of these patients and two controls had serum antibodies to the *intimin"**-*binding"** region of Tit, whereas none of the sera contained antibodies *binding"** to either of the intracellular domains of *Tir"**, By immunoblotting, 10 of 14 culture-positive patients had antibodies to the conserved region of *intimin"**, eight of whom were infected with E, coli O157 phage type 2, Thirty-six of 60 sera from culture-negative but E coli O157 LPS antibody-positive patients had antibodies to *intimin"** as determined by ELISA, The secreted *proteins"** are expressed in vivo during infection and are considered as pathogenic markers. Antibodies to these *proteins"** may form the basis of a serodiagnostic test for the detection of patients infected with VTEC which carry the locus for the enterocyte effacement pathogenicity island and provide an adjunct test to the established serological tests based on VTEC LPS.

11216985 GENUINE ARTICLE#: 268UL NUMBER OF REFERENCES: 27

TITLE: Hierarchy in the expression of the locus of enterocyte effacement genes of *enteropathogenic"** Escherichia *coli"**
AUTHOR(S): Friedberg D; Umanski T; Fang YA; Rosenshine I (REPRINT)
AUTHOR(S) E-MAIL: ilanro@cc.huji.ac.il
CORPORATE SOURCE: Hebrew Univ Jerusalem, Dept Mol Genet, POB 12272/IL-91120 Jerusalem//Israel/ (REPRINT); Hebrew Univ Jerusalem, Dept Mol Genet, /IL-91120 Jerusalem//Israel/; Hebrew Univ Jerusalem, Dept Biotechnol, /IL-91120 Jerusalem//Israel/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V34, N5 (DEC), P941-952
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (*EPEC"**) elicit changes in host cell morphology and cause actin rearrangement, a phenotype that has commonly been referred to as *attaching"**/ *effacing"** (AE) lesions. The ability of *EPEC"** to induce AE lesions is dependent upon a type III *protein"** secretion/translocation system that is encoded by genes clustered in a 35.6 kb DNA segment, named the locus of enterocyte effacement (LEE). We used transcriptional fusions between the green fluorescent *protein"** (gfp) reporter gene and LEE genes rorf2, orf3, orf5, escJ, escV and eae, together with immunoblot analysis with antibodies against *Tir"**, *intimin"**, EspB and EspF, to analyse the genetic regulation of the LEE. The expression of all these LEE genes was strictly dependent upon the presence of a functional integration host factor (IHF). IHF *binds"** specifically upstream from the ler (orf1) promoter and appears to activate expression of ler, orf3, orf5 and rorf2 directly. The ler-encoded Ler *protein"** was involved in activating the expression of escJ, escV, *tir"**, eae, espB and espF. Expression of both IHF and Ler was needed to elicit actin rearrangement associated with AE lesions. In conclusion, IHF directly activates the expression of the ler and rorf2 transcriptional units, and Ler in turn mediates the expression of the other LEE genes.

7/3,AB/27 (Item 15 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

11013552 GENUINE ARTICLE#: 245EX NUMBER OF REFERENCES: 60
TITLE: The *Tir"**-*binding"** region of *enterohaemorrhagic"** Escherichia *coli"** *intimin"** is sufficient to trigger actin condensation after bacterial-induced host cell signalling
AUTHOR(S): Liu H; Magoun L; Luperchio S; Schauer DB; Leong JM (REPRINT)
AUTHOR(S) E-MAIL: john.leong@umassmed.edu
CORPORATE SOURCE: Univ Massachusetts, Dept Mol Genet & Microbiol, 55 Lake

Ave N/Worcester//MA/01655 (REPRINT); Univ Massachusetts, Dept Mol Genet & Microbiol, /Worcester//MA/01655; MIT, Dept Bioengn & Environm Hlth, /Cambridge//MA/02139; MIT, Div Comparat Med, /Cambridge//MA/02139
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V34, N1 (OCT), P67-81
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enterohaemorrhagic"** Escherichia *coli"** (*EHEC"**) has emerged as an important agent of diarrhoeal disease. Attachment to host cells, an essential step during intestinal colonization by *EHEC"**, is associated with the formation of a highly organized cytoskeletal structure containing filamentous actin, termed an *attaching"** and *effacing"** (A/E) lesion, directly beneath bound bacteria. The outer membrane *protein"** *intimin"** is required for the formation of this structure, as is *Tir"**, a bacterial *protein"** that is translocated into the host cell and is thought to function as a receptor for *intimin"**, To understand *intimin"** function better, we fused *EHEC"** *intimin"** to a homologous *protein"**, Yersinia pseudotuberculosis invasin, or to maltose-*binding"** *protein"**. The N-terminal 539 amino acids of *intimin"** were sufficient to promote outer membrane localization of the C-terminus of invasin and, conversely, the N-terminal 489 amino acids of invasin were sufficient to promote the localization of the C-terminus of *intimin"**, The C-terminal 181 residues of *intimin"** were sufficient to *bind"** mammalian cells that had been preinfected with an *enteropathogenic"** E. *coli"** strain that expresses *Tir"** but not *intimin"**. *Binding"** of *intimin"** derivatives to preinfected cells correlated with *binding"** to recombinant *Tir"** *protein"**. Finally, the 181-residue minimal *Tir"**-*binding"** region of *intimin"**, when purified and immobilized on latex beads, was sufficient to trigger A/E lesions on preinfected mammalian cells.

7/3,AB/28 (Item 16 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

10970744 GENUINE ARTICLE#: 240GP NUMBER OF REFERENCES: 47
TITLE: Identification of CesT, a chaperone for the type III secretion of *Tir"** in *enteropathogenic"** Escherichia *coli"**
AUTHOR(S): Elliott SJ; Hutcheson SW; Dubois MS; Mellies JL; Wainwright LA; Batchelor M; Frankel G; Knutton S; Kaper JB (REPRINT)
AUTHOR(S) E-MAIL: jkaper@umaryland.edu
CORPORATE SOURCE: Univ Maryland, Ctr Vaccine Dev, 685 W Baltimore St/Baltimore//MD/21201 (REPRINT); Univ Maryland, Ctr Vaccine Dev, /Baltimore//MD/21201; Univ Maryland, Dept Microbiol & Immunol,

/Baltimore//MD/21201; Univ Maryland, Dept Mol Genet & Cell Biol, /College Pk//MD/20742; Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Univ Birmingham, Inst Child Hlth, /Birmingham B16 8ET/W Midlands/England/

PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V33, N6 (SEP), P1176-1189
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The locus of enterocyte effacement of *enteropathogenic"** Escherichia *coli"** encodes a type III secretion system, an outer membrane *protein"** adhesin (*intimin"**, the product of eae) and *Tir"**, a translocated *protein"** that becomes a host cell receptor for *intimin"**. Many type III secreted *proteins"** require chaperones, which function to stabilize *proteins"**, prevent inappropriate *protein"**-*protein"** interactions and aid in secretion. An open reading frame located between fir and eae, previously named orfU, was predicted to encode a *protein"** with partial similarity to the Yersinia SycH chaperone. We examined the potential of the orfU gene product to serve as a chaperone for *Tir"**. The orfU gene encoded a 15 kDa cytoplasmic *protein"** that specifically interacted with *Tir"** as demonstrated by the yeast two-hybrid assay, column *binding"** and coimmunoprecipitation experiments. An orfU mutant was defective in *attaching"**-*effacing"** lesion formation and *Tir"** secretion, but was unaffected in expression of other virulence factors. OrfU:appeared to stabilize *Tir"** levels in the cytoplasm, but was not absolutely necessary for secretion of *Tir"**. Based upon the physical similarities, phenotypic characteristics and the demonstrated interaction with *Tir"**, orfU is redesignated as cesT for the chaperone for E. coli secretion of *Tir"** .

7/3,AB/29 (Item 17 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

10970743 GENUINE ARTICLE#: 240GP NUMBER OF REFERENCES: 57
TITLE: *Enteropathogenic"** Escherichia *coli"** *translocated"** *intimin"** *receptor"**, *Tir"**, requires a specific chaperone for stable secretion
AUTHOR(S): Abe A; de Grado M; Pfuetzner RA; Sanchez-SanMartin C; DeVinney R ; Puente JL; Strynadka NCJ; Finlay BB (REPRINT)
AUTHOR(S) E-MAIL: bfinlay@unixg.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, Room 237,Wesbrook Bldg,6174 Univ Blvd/Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/; Univ

British Columbia, Dept Biochem & Mol Biol, /Vancouver/BC V6T 1Z3/Canada/; Kitasato Inst, Minato Ku, /Tokyo 108//Japan/; Univ Nacl Autonoma Mexico, Dept Mol Microbiol, /Cuernavaca 62250/Morelos/Mexico/

PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V33, N6 (SEP), P1162-1175
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (*EPEC"**) secretes several Esps (E. coli-secreted *proteins"**) that are required for full virulence. Insertion of the bacterial *protein"** *Tir"** into the host epithelial cell membrane is facilitated by a type III secretion apparatus, and at least EspA and EspB are required for *Tir"** translocation. An *EPEC"** outer membrane *protein"**, *intimin"**, interacts with *Tir"** on the host membrane to establish intimate attachment and formation of a pedestal-like structure. In this study, we identified a *Tir"** chaperone, CesT, whose gene is located between *tir"** and eae (which encodes *intimin"**). A mutation in cesT abolished *Tir"** secretion into culture supernatants and significantly decreased the amount of *Tir"** in the bacterial cytoplasm. In contrast, this mutation did not affect the secretion of the Esp *proteins"**. The level of *tir"** mRNA was not affected by the cesT mutation, indicating that CesT acts at the post-transcriptional level. The cesT mutant could not induce host cytoskeletal rearrangements, and displayed the same phenotype as the fir mutant. Gel overlay and GST pulldown assays demonstrated that CesT specifically interacts with *Tir"**, but not with other Esp *proteins"**. Furthermore, by using a series of *Tir"** deletion derivatives, we determined that the CesT *binding"** domain is located within the first 100 amino-terminal residues of *Tir"**, and that the pool of *Tir"** in the bacterial cytoplasm was greatly reduced when this domain was disrupted. Interestingly, this domain was not sufficient for *Tir"** secretion, and at least the first 200 residues of *Tir"** were required for efficient secretion. Gel filtration studies showed that *Tir"**-CesT forms a large multimeric complex. Collectively, these results indicate that CesT is a *Tir"** chaperone that may act as an anti-degradation factor by specifically *binding"** to its amino-terminus, forming a multimeric stabilized complex.

7/3,AB/30 (Item 18 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

10910288 GENUINE ARTICLE#: 234HF NUMBER OF REFERENCES: 46
TITLE: A novel chromosomal locus of *enteropathogenic"** Escherichia *coli"** (*EPEC"**), which encodes a bfpT-regulated chaperone-like

*protein"**, TrcA, involved in microcolony formation by *EPEC"**
AUTHOR(S): Tobe T (REPRINT); Tatsuno I; Katayama E; Wu CY; Schoolnik GK; Sasakawa C
AUTHOR(S) E-MAIL: torutobe@ims.u-tokyo.ac.jp
CORPORATE SOURCE: Univ Tokyo, Minato Ku, 4-6-1 Shirokanedai/Tokyo 1080071//Japan/ (REPRINT); Univ Tokyo, Minato Ku, /Tokyo 1080071//Japan/; Univ Tokyo, Minato Ku, /Tokyo 1080071//Japan/; Stanford Univ, Sch Med, /Stanford//CA/94305; Osaka Univ, Dept Bacterial Toxinol, /Suita/Osaka 565/Japan/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V33, N4 (AUG), P741-752
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The bfpTVW operon, also known as the per operon, of *enteropathogenic"** Escherichia *coli"** (*EPEC"**) is required for the transcriptional activation of the bfp operon, which encodes the major subunit and assembly machinery of bundle-forming pill (BFP). An immobilized T7-tagged BfpT fusion *protein"** that *binds"** specifically to upstream promoter sequences of bfpA and eae was used to 'fish out' from a promoter library other *EPEC"** chromosomal fragments that are bound by the BfpT *protein"**. After screening for promoters exhibiting bfpTVW-dependent expression, one was identified that was positively regulated by bfpTVW and that is not present in the chromosomes of two non-virulent E. coli laboratory strains, DH5 alpha and HB101. Further analysis of this positively regulated promoter in *EPEC"** showed that it resided within a 4.9 kb sequence that is not present in E. coli K12. This locus, located downstream of the potB gene, was found to contain four open reading frames (ORFs): bfpTVW-activated promoter was localized upstream of ORF1. An ORF1 knockout mutant produced less of the BFP structural subunit (BfpA) and formed smaller than normal adherent microcolonies on cultured epithelial cells; however, this mutation did not affect bfp transcription. An ORF1-His6 fusion *protein"** specifically bound the preprocessed and mature forms of the BfpA *protein"** and thus appears to stabilize the former within the cytoplasmic compartment. ORF1 therefore is a newly isolated *EPEC"** chromosomal gene that encodes a chaperone-like *protein"** involved in the production of BFP. Hence, ORF1 was designated trcA (bfpT-regulated chaperone-like *protein"** gene). The TrcA *protein"** also specifically bound 39 kDa and *90"** *kDa"** *proteins"** that are expressed by *EPEC"** but not by E. coli K12. The *90"** *kDa"** *protein"** was revealed to be *intimin"**, a *protein"** product of the eae gene, which is required for the *EPEC"** *attaching"**/*effacing"** phenotype, suggesting a direct interaction of TrcA with *intimin"** in the cytoplasmic compartment.

7/3,AB/31 (Item 19 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

10749914 GENUINE ARTICLE#: 219LV NUMBER OF REFERENCES: 24
TITLE: Role of bacterial *intimin"** in colonic hyperplasia and inflammation
AUTHOR(S): Higgins LM (REPRINT); Frankel G; Connerton I; Goncalves NS; Dougan G; MacDonald TT
CORPORATE SOURCE: St Bartholomews & Royal London Sch Med & Dent, Dept Paediat Gastroenterol, /London EC1A 7BE//England/ (REPRINT); St Bartholomews & Royal London Sch Med & Dent, Dept Paediat Gastroenterol, /London EC1A 7BE//England/; Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Univ Nottingham, Div Food Sci, /Loughborough LE12 5RD/Leics/England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: SCIENCE, 1999, V285, N5427 (JUL 23), P588-591
PUBLISHER: AMER ASSOC ADVANCEMENT SCIENCE, 1200 NEW YORK AVE, NW, WASHINGTON, DC 20005 USA
ISSN: 0036-8075
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (*EPEC"**) cells adhere to gut epithelial cells through *intimin"** alpha: the ligand for a bacterially derived epithelial transmembrane *protein"** called the *translocated"** *intimin"** *receptor"**, Citrobacter rodentium colonizes the mouse colon in a similar fashion and uses a different *intimin"**: *intimin"** beta. *Intimin"** alpha was found to costimulate submitogenic signals through the T cell receptor. Dead *intimin"** beta(+) C. rodentium, *intimin"** a-transfected C. rodentium or E. coli strain K12, and *EPEC"** induced mucosal hyperplasia identical to that caused by C. rodentium live infection, as well as a massive T helper cell-type 1 immune response in the colonic mucosa, Mutation of cysteine-937 of *intimin"** to alanine reduced costimulatory activity in vitro and prevented immunopathology in vivo. The mucosal changes elicited by C. rodentium were interferon-gamma-dependent. Immunopathology induced by *intimin"** enables the bacteria to promote conditions that are favorable for increased microbial colonization.

7/3,AB/32 (Item 20 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

10475977 GENUINE ARTICLE#: 187BW NUMBER OF REFERENCES: 26
TITLE: *Binding"** of *intimin"** from *enteropathogenic"** Escherichia *coli"** to *Tir"** and to host cells
AUTHOR(S): Hartland EL; Batchelor M; Delahay RM; Hale C; Matthews S; Dougan

G; Knutton S; Connerton I; Frankel G (REPRINT)
AUTHOR(S) E-MAIL: g.frankel@ic.ac.uk
CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Univ London Imperial Coll Sci Technol & Med, Ctr Struct Biol, /London SW7 2AZ//England/; Univ Birmingham, Inst Child Hlth, /Birmingham B4 6NH/W Midlands/England/; Inst Food Res, Reading Lab, /Reading RG6 6BZ/Berks/England/; Univ Nottingham, Div Food Sci, /Loughborough LE12 5RD/Leics/England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V32, N1 (APR), P151-158
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (*EPEC"**) induce characteristic *attaching"** and *effacing"** (A/E) lesions on epithelial cells. This event is mediated, in part, by *binding"** of the bacterial outer membrane *protein"**, *intimin"**, to a second *EPEC"** *protein"**, *Tir"** (*translocated"** *intimin"** *receptor"**), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study, we have localized the *intimin"**-*binding"** domain of *Tir"** to a central 107-amino-acid region, designated *Tir"**-M. We provide evidence that both the amino- and carboxy-termini of *Tir"** are located within the host cell. In addition, using immunogold labelling electron microscopy, we have confirmed that *intimin"** can *bind"** independently to host cells even in the absence of *Tir"**, This *Tir"**-independent interaction and the ability of *EPEC"** to induce A/E lesions requires an intact lectinlike module residing at the carboxy-terminus of the *intimin"** *polypeptide"**. Using the yeast two-hybrid system and gel overlays, we show that *intimin"** can *bind"** both *Tir"** and *Tir"**-M even when the lectin-like domain is disrupted. These data provide strong evidence that *intimin"** interacts not only with *Tir"** but also in a lectinlike manner with a host cell *intimin"** receptor.

7/3,AB/33 (Item 21 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

10430360 GENUINE ARTICLE#: 182MT NUMBER OF REFERENCES: 46
TITLE: Structure of the cell-adhesion fragment of *intimin"** from *enteropathogenic"** Escherichia *coli"**
AUTHOR(S): Kelly G; Prasannan S; Daniell S; Fleming K; Frankel G; Dougan G; Connerton I; Matthews S (REPRINT)
AUTHOR(S) E-MAIL: s.j.matthews@ic.ac.uk

CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biochem, Exhibit Rd/London SW7 2AY//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AY//England/; Univ London Imperial Coll Sci Technol & Med, Ctr Struct Biol, /London SW7 2AY//England/; AFRC, Reading Lab, /Reading RG6 6BZ/Berks/England/; Univ London Imperial Coll Sci Technol & Med, Wellcome Ctr Infect DIs, /London SW7 2AY//England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: NATURE STRUCTURAL BIOLOGY, 1999, V6, N4 (APR), P313-318
PUBLISHER: NATURE AMERICA INC, 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707 USA
ISSN: 1072-8368
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (*EPEC"**) induce gross cytoskeletal rearrangement within epithelial cells, immediately beneath the attached bacterium. The C-terminal 280 amino acid residues of *intimin"** (Int280; 30.1 kDa), a bacterial cell-adhesion molecule, mediate the intimate bacterial host-cell interaction. Recently, interest in this process has been stimulated by the discovery that the bacterial *intimin"** receptor *protein"** (*Tir"**) is translocated into the host cell membrane, phosphorylated, and after *binding"** *intimin"** triggers the intimate attachment. Using multidimensional nuclear magnetic resonance (NMR) and combining perdeuteration with site-specific protonation of methyl groups, we have determined the global fold of Int280. This represents one of the largest, non-oligomeric *protein"** structures to be determined by NMR that has not been previously resolved by X-ray crystallography, Int280 comprises three domains; two immunoglobulin-like domains and a C-type lectinlike module, which define a new family of bacterial adhesion molecules. These findings also imply that carbohydrate recognition may be important in *intimin"**-mediated cell adhesion.

7/3,AB/34 (Item 22 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

10323436 GENUINE ARTICLE#: 170EB NUMBER OF REFERENCES: 33
TITLE: Phosphorylation of tyrosine 474 of the *enteropathogenic"** Escherichia *coli"** (*EPEC"**) *Tir"** receptor molecule is essential for actin nucleating activity and is preceded by additional host modifications
AUTHOR(S): Kenny B (REPRINT)
AUTHOR(S) E-MAIL: B.Kenny@bristol.ac.uk
CORPORATE SOURCE: Univ Bristol, Dept Pathol & Microbiol, Univ Walk/Bristol BS8 1TD/Avon/England/ (REPRINT); Univ Bristol, Dept Pathol & Microbiol, /Bristol BS8 1TD/Avon/England/
PUBLICATION TYPE: JOURNAL

PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V31, N4 (FEB), P1229-1241
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ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The *enteropathogenic"** Escherichia *coli"** (*EPEC"**) *Tir"** *protein"** becomes tyrosine phosphorylated in host cells and displays an increase in apparent molecular mass. The interaction of *Tir"** with the *EPEC"** outer membrane *protein"**, *intimin"**, triggers actin nucleation beneath the adherent bacteria. The *enterohaemorrhagic"** E. *coli"** 0157:H7 (*EHEC"**) *Tir"** molecule is not tyrosine phosphorylated. In this paper, *Tir"** tyrosine phosphorylation is shown to be essential for actin nucleation activity, but not for the increase in apparent molecular mass observed in target cells. Tyrosine phosphorylation had no role in *Tir"** molecular mass shift, indicating additional host modifications. Analysis of *Tir"** intermediates indicates that tyrosine-independent modification functions to direct *Tir"**'s correct insertion from the cytoplasm into the host membrane. Deletion analysis identified *Tir"** domains participating in translocation, association with the host membrane, modification and antibody recognition. *Intimin"** was found to *bind"** a 55-amino-acid region (TIBA) within *Tir"** that topological and sequence analysis suggests is located in an extracellular loop. Homologous TIBA sequences exist in integrins, which also *bind"** *intimin"**. Collectively, this study provides definitive evidence for the importance of tyrosine phosphorylation for *EPEC"** *Tir"** function and reveals differences in the pathogenicity of *EPEC"** and *EHEC"**, The data also suggest a mechanism for *Tir"** insertion into the host membrane, as well as providing clues to the mode of *intimin"**-integrin interaction.

7/3,AB/35 (Item 23 from file: 440)

08563608 GENUINE ARTICLE#: XF631 NUMBER OF REFERENCES: 30
TITLE: *Intimin"**-dependent *binding"** of *enteropathogenic"** Escherichia *coli"** to host cells triggers novel signaling events, including tyrosine phosphorylation of phospholipase C-gamma 1
AUTHOR(S): Kenny B; Finlay BB (REPRINT)
CORPORATE SOURCE: UNIV BRITISH COLUMBIA,BIOTECHNOL LAB, ROOM 237 WESBROOK BLDG, 6174 UNIV BLVD/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV BRITISH COLUMBIA,BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1997, V65, N7 (JUL), P2528-2536
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0019-9567

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (*EPEC"**) interactions with HeLa epithelial cells induced the tyrosine phosphorylation of a host *protein"** of approximately 150 kDa, Hp150. Phosphorylation of this *protein"** hand was dependent on the interaction of the *EPEC"** *protein"** *intimin"** with epithelial cell surfaces and was correlated with pedestal formation, Hp150 phosphorylation was specifically inhibited by the addition of cytochalasin D, an inhibitor of actin polymerization, although this appeared to be an indirect effect preventing interaction of *intimin"** with its receptor, tyrosine-phosphorylated *Hp90"**, and thus triggering Hp150 phosphorylation. This suggests the involvement of an actin-based movement of membrane-bound tyrosine-phosphorylated *Hp90"** to allow its interaction with *intimin"**. Analysis of the tyrosine-phosphorylated Hp150 *protein"** demonstrated that it is heterogeneous in composition, with phospholipase C-gamma 1 (PLC-gamma 1) being a minor component. Activation of PLC-gamma 1 by tyrosine phosphorylation leads to inositol triphosphate and Ca2+ fluxes, events defected following *EPEC"** infection. *EPEC"** also induced tyrosine dephosphorylation of host *proteins"**, including a 240-kDa host *protein"** (Hp240), following *EPEC"** infection, *Protein"** dephosphorylation appears to be a signaling event which occurs independently of *intimin"**. Inhibition of host tyrosine dephosphorylation events by the addition of the tyrosine phosphatase inhibitor sodium vanadate did not pl:event actin accumulation beneath the adherent bacteria. We conclude that *EPEC"** induces two sets of signaling events following infection, One set is dependent on *EPEC"** *proteins"** secreted by the type III secretion pathway (EspA and EspB) which induces *Hp90"** tyrosine phosphorylation and dephosphorylation of host phosphotyrosine *proteins"**. The second set, which is also dependent on the first signaling events, requires *intimin"** interaction with its receptor, tyrosine-phosphorylated *Hp90"**, to trigger Hp150 and PLC-gamma 1 tyrosine phosphorylation as well as pedestal formation. Inhibition of pedestal formation by tyrosine kinase inhibitors indicates an important role for tyrosine phosphorylation events during *EPEC"** subversion of host processes.

7/3,AB/36 (Item 24 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

07453860 GENUINE ARTICLE#: UQ620 NUMBER OF REFERENCES: 33
TITLE: A PATHOGENIC BACTERIUM TRIGGERS EPITHELIAL SIGNALS TO FORM A FUNCTIONAL BACTERIAL RECEPTOR THAT MEDIATES ACTIN PSEUDOPOD FORMATION
AUTHOR(S): ROSENSHINE I; RUSCHKOWSKI S; STEIN M; REINSCHEID DJ; MILLS SD; FINLAY BB (Reprint)
CORPORATE SOURCE: UNIV BRITISH COLUMBIA,BIOTECHNOL LAB/VANCOUVER/BC V6T

1Z3/CANADA/ (Reprint); UNIV BRITISH COLUMBIA,BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/; UNIV BRITISH COLUMBIA,DEPT BIOCHEM & MOLEC BIOL/VANCOUVER/BC V6T 1Z3/CANADA/; UNIV BRITISH COLUMBIA,DEPT MICROBIOL & IMMUNOL/VANCOUVER/BC V6T 1Z3/CANADA/; HEBREW UNIV JERUSALEM,FAC MED,DEPT BIOTECHNOL &MOL GENET/IL-91120 JERUSALEM//ISRAEL/

PUBLICATION: EMBO JOURNAL, 1996, V15, N11 (JUN 3), P2613-2624
ISSN: 0261-4189
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic"** E. *coli"** (*EPEC"**) belongs to a group of bacterial pathogens that induce actin accumulation beneath adherent bacteria. We found that *EPEC"** adherence to epithelial cells mediates the formation of fingerlike pseudopods (up to 10 mu m) beneath bacteria. These actin-rich structures also contain tyrosine phosphorylated host *proteins"** concentrated at the pseudopod tip beneath adherent *EPEC"**. Intimate bacterial adherence (and pseudopod formation) occurred only after prior bacterial induction of tyrosine phosphorylation of an epithelial membrane *protein"**, *Hp90"**, which then associates directly with an *EPEC"** adhesin, *intimin"**. These interactions lead to cytoskeletal nucleation and pseudopod formation. This is the first example of a bacterial pathogen that triggers signals in epithelial cells which activates receptor *binding"** activity to a specific bacterial ligand and subsequent cytoskeletal rearrangement.

7/3,AB/37 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0239735 DBA Accession No.: 1999-09836 PATENT
Escherichia coli recombinant *intimin"** receptor *protein"** - useful for distinguishing between enteropathogenic and enterohemorrhagic infection and for therapy and diagnosis

AUTHOR: Finlay B B; Kenny B; Devinney R; Stein M
CORPORATE SOURCE: Vancouver, British Columbia, Canada.
PATENT ASSIGNEE: Univ.British-Columbia 1999

PATENT NUMBER: WO 9924576 PATENT DATE: 19990520 WPI ACCESSION NO.: 1999-337712 (1928)
PRIORITY APPLIC. NO.: US 65130 APPLIC. DATE: 19971112
NATIONAL APPLIC. NO.: WO 98CA1042 APPLIC. DATE: 19981110
LANGUAGE: English

ABSTRACT: A translocated Escherichia coli *intimin"** receptor *protein"** (I) that *binds"** *intimin"** is new. Also claimed are: a DNA sequence (II) encoding (I) and its complements, fragments and variants; DNA probes specific for (II); vectors encoding (II) and host cells containing them; (I)-specific polyclonal or monoclonal antibody; recombinant production of (I); a fusion *protein"** containing (I); a method for identifying modulators of (I); a method for differentiating

between *attaching"** and *effacing"** pathogens by contacting them with an anti-(I) antibody and an anti-phosphotyrosine antibody; drug delivery to (I)-containing cells using a cell delivery vehicle; kits for the detection of (I) and (II); and a method for inducing a cell-mediated immune response in cattle or humans to a *protein"** of interest by contacting a subject with an attenuated bacteria, where the bacterium lacks an EspA or EspB *protein"**, and contains (II) in a fusion construct. The presence of (I) in a sample is indicative of enteropathogenic or enterohemorrhagic infection. (91pp)

- Author(s)

Set	Items	Description
S8	735	AU=(FINLAY, B? OR FINLAY B?)
S9	268	AU=(KENNY, B? OR KENNY B?)
S10	33	AU=(DEVINNEY, R? OR DE VINNEY, R? OR DEVINNEY R? OR DE VINNEY R?)
S11	3355	AU=(STEIN, M? OR STEIN M?)
S12	4	S8 AND S9 AND S10 AND S11
S13	67	S8 AND (S9 OR S10 OR S11)
S14	18	S9 AND (S10 OR S11)
S15	7	S10 AND S11
S20	52	(S8 OR S9 OR S10 OR S11 OR S13) AND S3
S21	42	(S12 OR S14 OR S15 OR S20) NOT S6
S22	22	RD (unique items)

>>>No matching display code(s) found in file(s): 65, 113

22/3,AB/1 (Item 1 from file: 65)
DIALOG(R)File 65:Inside Conferences

02302103 INSIDE CONFERENCE ITEM ID: CN024112431
Molecular mechanisms of enteropathogenic E. coli: Signal transduction, pedestal formation, intimate contact, and diarrhea
Finlay, B. B.; *Kenny, B."**; *Stein, M."**; Reinscheid, D.
CONFERENCE: Enteropathogenic Escherichia coli-International symposium
REVISTA DE MICROBIOLOGIA, 1996; VOL 27; SUPP 1 P: 95-98
(np), 1996
ISSN: 0001-3714
LANGUAGE: English DOCUMENT TYPE: Conference Papers
CONFERENCE EDITOR(S): Kaper, J. B.
CONFERENCE LOCATION: Sao Paulo, Brazil
CONFERENCE DATE: Aug 1995 (199508) (199508)

22/3,AB/2 (Item 2 from file: 65)
DIALOG(R)File 65:Inside Conferences

01741681 INSIDE CONFERENCE ITEM ID: CN017738833
Enteropathogenic E. coli Exploitation of Host Epithelial Cells
Finlay, B. B.; Ruschkowski, S.; *Kenny, B."**; *Stein, M."**
CONFERENCE: Microbial pathogenesis and immune response-Meeting; 2nd
ANNALS- NEW YORK ACADEMY OF SCIENCES, 1996; VOL 797 P: 26-31
New York Academy of Sciences, 1996
ISSN: 0077-8923 ISBN: 1573310166; 1573310174
LANGUAGE: English DOCUMENT TYPE: Conference Papers
CONFERENCE EDITOR(S): Ades, E. W.; Morse, S. A.; Rest, R. F.
CONFERENCE LOCATION: New York, NY
CONFERENCE DATE: Oct 1995 (199510) (199510)

22/3,AB/3 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

14307127 PASCAL No.: 99-0513847
Type III secretion-dependent hemolytic activity of enteropathogenic Escherichia coli
WARAWA J; *FINLAY B B"**; *KENNY B"**
Department of Pathology and Microbiology, School of Medical Sciences, Bristol, United Kingdom; Biotechnology Laboratory, Vancouver, British Columbia, V6T 1Z3, Canada
Journal: Infection and immunity, 1999, 67 (10) 5538-5540
Language: English
Enteropathogenic Escherichia coli (EPEC) was found to exhibit a type III secretion-dependent, contact-mediated, hemolytic activity requiring the EspA, EspB, and EspD secreted *proteins"**. EspB and EspD display homology to pore-forming molecules. Our data suggest a mechanism to explain the requirement for all three Esp *proteins"** in the transfer of EPEC *proteins"**, such as *Tir"**, into target cells.

22/3,AB/4 (Item 2 from file: 144)
DIALOG(R)File 144:Pascal

14080585 PASCAL No.: 99-0273444
Enteropathogenic Escherichia coli : cellular harassment
Host-microbe interactions: bacteria
*DEVINNEY R"**; KNOECHEL D G; *FINLAY B B"**
COSSART Pascale, ed; MILLER Jeff F, ed
Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada
Unite des Interations Bacteries-Cellules, Institut Pasteur, 28 rue du Dr

Roux, 75015 Paris, France; University of California Los Angeles School of Medicine, Dept of Microbiology and Immunology, 10833 Le Conte Ave., Los Angeles, CA 90024, United States

Journal: Current opinion in microbiology, 1999, 2 (1) 83-88

Language: English

The mechanisms by which enteropathogenic Escherichia coli (EPEC) mediates diarrhea remain a mystery. Recently a number of interesting and at times surprising results have come from studying EPEC interactions with host cells. Identification and characterization of bacterial factors, including *Tir"**, EspA, EspB and EspD, and host responses have expanded our grasp of the diverse effects of EPEC on host cells.

22/3,AB/5 (Item 3 from file: 144)
DIALOG(R)File 144:Pascal

13217033 PASCAL No.: 97-0484125

Characterization of two virulence proteins secreted by rabbit enteropathogenic Escherichia coli, EspA and EspB, whose maximal expression is sensitive to host body temperature

ABE A; *KENNY B"**; *STEIN M"**; FINLAY B B

Biotechnology, Laboratory, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada; Department of Bacteriology, The Kitasato Institute, Minato-ku, Tokyo 108, Japan

Journal: Infection and immunity, 1997, 65 (9) 3547-3555

Language: English

Enteropathogenic Escherichia coli (EPEC) and rabbit EPEC (RDEC-1) cause unique histopathological features on intestinal mucosa, including attaching/effacing (A/E) lesions. Due to the human specificity of EPEC, RDEC-I has been used as an animal model to study EPEC pathogenesis. At least two of the previously identified EPEC-secreted proteins, EspA and EspB, are required for triggering host epithelial signal transduction pathways, intimate adherence, and A/E lesions. However, the functions of these secreted proteins and their roles in pathogenesis have not been characterized. To investigate the function of EspA and EspB in RDEC-1, the espA and espB genes were cloned and their sequences were compared to that of EPEC 0127. The EspA proteins showed high similarity (88.5% identity), while EspB was heterogeneous in internal regions (69.8% identity). However, RDEC-I EspB was identical to that of enterohemorrhagic E. coli serotype 026. Mutations in RDEC-I espA and espB revealed that the corresponding RDEC-I gene products are essential for triggering of host signal transduction pathways and invasion into HeLa cells. Complementation with plasmids containing EPEC espA or/and espB genes into RDEC-I mutant strains demonstrated that they were functionally interchangeable, although the EPEC proteins mediated higher levels of invasion. Furthermore, maximal

expression of RDEC-1 and EPEC-secreted proteins occurred at their respective host body temperatures, which may contribute to the lack of EPEC infectivity in rabbits.

22/3,AB/6 (Item 4 from file: 144)
DIALOG(R)File 144:Pascal

13078049 PASCAL No.: 97-0369601
Enteropathogenic Escherichia coli protein secretion is induced in response to conditions similar to those in the gastrointestinal tract
*KENNY B"**; ABE A; *STEIN M"**; FINLAY B B
Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, V6T-1Z3, Canada
Journal: Infection and immunity, 1997, 65 (7) 2606-2612
Language: English
The pathogenicity of enteropathogenic Escherichia coli (EPEC) is associated with the expression and secretion of specific bacterial factors. EspB is one such secreted protein which is required to trigger host signaling pathways resulting in effacement of microvilli and cytoskeletal rearrangements. These events presumably contribute to the ensuing diarrhea associated with EPEC infections. EPEC encounters several environmental changes and stimuli during its passage from the external environment into the host gastrointestinal tract. In this paper we show that the secretion of EspB is subject to environmental regulation, and maximal secretion occurs under conditions reminiscent of those in the gastrointestinal tract. Thus, secretion is maximal at 37 Degree C, pH 7, and physiological osmolarity. In addition, maximal secretion requires the presence of sodium bicarbonate and calcium and is stimulated by millimolar concentrations of Fe(NO SUB 3) SUB 3 . The secretion of the four other EPEC-secreted proteins appears to be modulated in a manner similar to that of EspB. Our results also show that secretion is not dependent on CO SUB z , as originally reported by Haigh et al. (FEMS Microbiol. Lett. 129: 63-67, 1995), but that CO SUB z more likely acts as a component of the medium buffering system, since CO SUB 2 dependence was abolished by the use of alternative buffers.

22/3,AB/7 (Item 5 from file: 144)
DIALOG(R)File 144:Pascal

10356299 PASCAL No.: 92-0559759

Signal transduction between enteropathogenic Escherichia coli (EPEC) and epithelial cells : EPEC induces tyrosine phosphorylation of host cell *proteins"** to initiate cytoskeletal rearrangement and bacterial uptake

ROSENSHINE I; DONNENBERG M S; KAPER J B; *FINLAY B B"**
Univ. British Columbia, Canadian Bacterial Diseases Network, biotechnology lab., Vancouver BC, Canada

Journal: EMBO journal, 1992, 11 (10) 3551-3560
Language: English

Upon attachment to cultured HeLa cells, enteropathogenic Escherichia coli (PEC) induces assembly of a complex cytoskeletal structure within the eucaryotic cell, localized beneath the afferent bacterium. In addition, EPEC induces its own internalization by non-phagocytic epithelial cells. We found that after binding to the epithelial cell surface, EPEC induces tyrosine phosphorylation of three eucaryotic *proteins"** . The major phosphorylation substrate is a *90"** *kDa"** *protein"** (*Hp90"**)

22/3,AB/8 (Item 1 from file: 440)

12568712 GENUINE ARTICLE#: 416HT NUMBER OF REFERENCES: 56
TITLE: Enteropathogenic Escherichia coli mediates antiphagocytosis through the inhibition of PI 3-kinase-dependent pathways
AUTHOR(S): Celli J; Olivier M; *Finlay BB (REPRINT)"**
AUTHOR(S) E-MAIL: bfinlay@interchange.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/; Univ Laval, Infect Dis Unit, /Quebec City/PQ G1V 4G2/Canada/
PUBLICATION TYPE: JOURNAL
PUBLICATION: EMBO JOURNAL, 2001, V20, N6 (MAR 15), P1245-1258
PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND
ISSN: 0261-4189
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The extracellular pathogen enteropathogenic Escherichia coli (EPEC) uses a type III secretion system to inhibit its uptake by macrophages. We show that EPEC antiphagocytosis is independent of the *translocated"** *intimin"** *receptor"** *Tir"** and occurs by preventing F-actin polymerization required for bacterial uptake. EPEC-macrophage contact triggered activation of phosphatidylinositol (PI) 3-kinase, which was subsequently inhibited in a type III secretion-dependent manner. Inhibition of PI 3-kinase significantly reduced uptake of a secretion-deficient mutant, without affecting antiphagocytosis by the wild type, suggesting that EPEC blocks a PI 3-kinase-dependent phagocytic pathway. EPEC specifically inhibited Fc gamma receptor- but not CR3-receptor mediated phagocytosis of opsonized zymosan, We showed that EPEC inhibits PI 3-kinase activity rather than

its recruitment to the site of bacterial contact. Phagocytosis of a secretion mutant correlated with the association of PI 3-kinase with tyrosine-phosphorylated *proteins"**, which wild-type EPEC prevented. These results show that EPEC blocks its uptake by inhibiting a PI 3-kinase-mediated pathway, and translocates effecters other than *Tir"** to interfere with actin-driven host cell processes. This constitutes a novel mechanism of phagocytosis avoidance by an extracellular pathogen.

22/3,AB/9 (Item 2 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12441711 GENUINE ARTICLE#: 404GZ NUMBER OF REFERENCES: 41
TITLE: Enteropathogenic Escherichia coli (EPEC) *Tir"** receptor molecule does not undergo full modification when introduced into host cells by EPEC-independent mechanisms
AUTHOR(S): *Kenny B (REPRINT)"**; Warawa J
AUTHOR(S) E-MAIL: B.Kenny@bristol.ac.uk
CORPORATE SOURCE: Sch Med Sci Bristol, Dept Pathol & Microbiol, Univ Walk/Bristol BS8 1TD/Avon/England/ (REPRINT); Sch Med Sci Bristol, Dept Pathol & Microbiol, /Bristol BS8 1TD/Avon/England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2001, V69, N3 (MAR), P1444-1453
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Enteropathogenic Escherichia coli (EPEC), like many other gram-negative pathogens, encodes a type III secretion apparatus dedicated to the release of virulence-associated *proteins"**. One such *protein"**, *Tir"**, is translocated into host cells, where it is modified by the addition of phosphate groups, resulting in a number of species with distinct molecular mass. One phosphorylation event, on tyrosine residue 474 of *Tir"**, does not contribute to shifts in molecular mass but is essential for its actin-nucleating function. The role of the nonphosphotyrosine related modifications is unknown. In this paper, we demonstrate, using three different approaches, that *Tir"** does not encode sufficient information to facilitate its complete modification when introduced into host cells in EPEC-independent mechanisms. Each system revealed that *Tir"** is a substrate for a host kinase whose action results in its partial modification to a form similar to one evident in EPEC-infected host cells. Further *Tir"** modification could not be induced by infecting cells with EPEC, suggesting that *Tir"** must be coexpressed with other EPEC factors to enable its full modification within host cells. One approach used Yersinia spp. to deliver *Tir"** into host cells, and

this system revealed that *Tir"** secretion and translocation can occur in the absence of the *Tir"** chaperone molecule, CesT (formerly known as OrfU). CesT was found to be an efficiency factor which was not required, unlike in EPEC, for *Tir"** stability, indicating that it may function to guide *Tir"** to the translocation apparatus or maintain it in a secretion-competent form.

22/3,AB/10 (Item 3 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12287537 GENUINE ARTICLE#: 387GA NUMBER OF REFERENCES: 43
TITLE: Targeting of an enteropathogenic Escherichia coli (EPEC) effector *protein"** to host mitochondria
AUTHOR(S): *Kenny B (REPRINT)"**; Jepson M
AUTHOR(S) E-MAIL: B.Kenny@bristol.ac.uk
CORPORATE SOURCE: Univ Bristol, Dept Pathol & Microbiol, Univ Walk/Bristol BS8 1TD/Avon/England/ (REPRINT); Univ Bristol, Dept Pathol & Microbiol, /Bristol BS8 1TD/Avon/England/; Univ Bristol, Cell Imaging Facil, /Bristol BS8 1TD/Avon/England/; Univ Bristol, Dept Biochem, /Bristol BS8 1TD/Avon/England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: CELLULAR MICROBIOLOGY, 2000, V2, N6 (DEC), P579-590
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 1462-5814
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Many Gram-negative pathogens use a type III secretion apparatus to deliver effector molecules into host cells to subvert cellular processes in favour of the pathogen. Enteropathogenic Escherichia coli (EPEC) uses such a system to deliver the *Tir"** effector molecule into host cells. In this paper, we show that the gene upstream of *tir"**, orf19, encodes an additional type III secreted effector *protein"**. Orf19 is delivered into host cells by a mechanism independent of endocytosis, but dependent on EspB. Orf19 is targeted to host mitochondria, where it appears to interfere with the ability to maintain membrane potential. Although the precise role of Orf19 remains to be elucidated, its interaction with mitochondria suggests a possible role in the subversion of key functions of these organelles, such as energy production or control of cell death. This is the first example of a type III secreted *protein"** targeted to mitochondria; it is probable that homologues (present in EPEC and Shigella species) and other bacterial effecters will also target this organelle.

22/3,AB/11 (Item 4 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12271566 GENUINE ARTICLE#: 384LF NUMBER OF REFERENCES: 24
TITLE: *Tir"** tyrosine phosphorylation and pedestal formation are delayed in enteropathogenic Escherichia coli sepZ :: TnphoA mutant 30-5-1(3)
AUTHOR(S): *DeVinney R"**; Nisan I; Ruschkowski S; Rosenshine I; *Finlay BB (REPRINT)"**
AUTHOR(S) E-MAIL: bfinlay@unixg.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/; Hebrew Univ Jerusalem, Dept Mol Genet, /IL-91120 Jerusalem//Israel/; Hebrew Univ Jerusalem, Dept Biotechnol, /IL-91120 Jerusalem//Israel/; Hebrew Univ Jerusalem, Dept Clin Microbiol, /IL-91120 Jerusalem//Israel/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2001, V69, N1 (JAN), P559-563
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Enteropathogenic Escherichia call (EPEC) strain 30-5-1(3) has been reported to form attaching and effacing (A/E) lesions without *Tir"** tyrosine phosphorylation. In this study, we show that 30-5-1(3), which has a transposon insertion within the sepZ gene, forms wild-type A/E lesions including *Tir"** tyrosine phosphorylation, but at a slower rate. A/E lesion formation by 30-5-1(3) occurs without detectable secretion of *Tir"** or other EPEC Esp secreted *proteins"**
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22/3,AB/12 (Item 5 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12265208 GENUINE ARTICLE#: 385NH NUMBER OF REFERENCES: 84
TITLE: Gut feelings: enteropathogenic E-coli (EPEC) interactions with the host
AUTHOR(S): Goosney DL (REPRINT); Gruenheid S; *Finlay BB"**
AUTHOR(S) E-MAIL: bfinlay@interchange.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1W5/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1W5/Canada/; Univ British Columbia, Dept Microbiol & Immunol, /Vancouver/BC V6T 1W5/Canada/
PUBLICATION TYPE: JOURNAL
PUBLICATION: ANNUAL REVIEW OF CELL AND DEVELOPMENTAL BIOLOGY, 2000, V16, P 173-+
PUBLISHER: ANNUAL REVIEWS, 4139 EL CAMINO WAY, PO BOX 10139, PALO ALTO, CA 94303-0139 USA

ISSN: 1081-0706
LANGUAGE: English DOCUMENT TYPE: REVIEW
ABSTRACT: Enteropathogenic Escherichia coli (EPEC) is a gram-negative bacterial pathogen that adheres to human intestinal epithelial cells, resulting in watery, persistent diarrhea. It subverts the host cell cytoskeleton, causing a rearrangement of cytoskeletal components into a characteristic pedestal structure underneath adherent bacteria. In contrast to other intracellular pathogens that affect the actin cytoskeleton from inside the host cytoplasm, EPEC remains extracellular and transmits signals through the host cell plasma membrane via direct injection of virulence factors by a "molecular syringe," the bacterial type III secretion system. One injected factor is *Tir"**, which functions as the plasma membrane receptor for EPEC adherence. *Tir"** directly links extracellular EPEC through the epithelial membrane and firmly anchors it to the host cell actin cytoskeleton, thereby initiating pedestal formation. In addition to stimulating actin nucleation and polymerization in the host cell, EPEC activates several other signaling pathways that lead to tight junction disruption, inhibition of phagocytosis, altered ion secretion, and immune responses. This review summarizes recent developments in our understanding of EPEC pathogenesis and discusses similarities and differences between EPEC pedestals, focal contacts, and Listeria monocytogenes actin tails.

22/3,AB/13 (Item 6 from file: 440)

10665713 GENUINE ARTICLE#: 209MM NUMBER OF REFERENCES: 122
TITLE: Enteropathogenic Escherichia coli: a pathogen that inserts its own receptor into host cells
AUTHOR(S): *DeVinney R"**; Gauthier A; Abe A; *Finlay BB (REPRINT)"**
AUTHOR(S) E-MAIL: bfinlay@unixg.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z4/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z4/Canada/; Kitasato Inst, Minato Ku, /Tokyo 108//Japan/
PUBLICATION TYPE: JOURNAL
PUBLICATION: CELLULAR AND MOLECULAR LIFE SCIENCES, 1999, V55, N6-7 (JUN), P 961-976
PUBLISHER: BIRKHAUSER VERLAG AG, VIADUKSTRASSE 40-44, PO BOX 133, CH-4010 BASEL, SWITZERLAND
ISSN: 1420-682X
LANGUAGE: English DOCUMENT TYPE: REVIEW
ABSTRACT: Enteropathogenic Escherichia coli (EPEC) is a major cause of infant diarrhea, killing hundreds of thousands of children per year worldwide. Intimate attachment to the host cell leading to the

formation of actin-rich pedestals beneath the adhering bacteria is an essential feature of EPEC pathogenesis. EPEC attaches to host cells via the outer membrane adhesin, intimin. It was recently shown that EPEC inserts its own receptor for intimate adherence, *Tir"** (*translocated"** *intimin"** *receptor"**) into the host cell membrane. The focus of this review is on the discovery and characterization of this novel receptor, and our current understanding of its role in pedestal formation. Gram-negative bacterial secretion systems, including type III secretion systems, are reviewed and discussed in the context of *Tir"** delivery into the host cell membrane. The relationship and relevance of in vitro models compared to the actual in vivo situation is essential to understanding disease. We have critically reviewed the use of animal models in studying EPEC infection. Elucidating the function of *Tir"** will contribute to our understanding of how EPEC mediates disease.

22/3,AB/14 (Item 7 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08996050 GENUINE ARTICLE#: YG492 NUMBER OF REFERENCES: 31
TITLE: Enteropathogenic E-coli (EPEC) transfers its receptor for intimate adherence into mammalian cells
AUTHOR(S): *Kenny B"**; *DeVinney R"**; *Stein M"**; Reinscheid DJ; Frey EA ; *Finlay BB (REPRINT)"**
CORPORATE SOURCE: UNIV BRITISH COLUMBIA,DEPT BIOCHEM & MOL BIOL, DEPT MICROBIOL & IMMUNOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV BRITISH COLUMBIA,DEPT BIOCHEM & MOL BIOL, DEPT MICROBIOL & IMMUNOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/
PUBLICATION TYPE: JOURNAL
PUBLICATION: CELL, 1997, V91, N4 (NOV 14), P511-520
PUBLISHER: CELL PRESS, 1050 MASSACHUSETTES AVE, CIRCULATION DEPT, CAMBRIDGE, MA 02138
ISSN: 0092-8674
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Enteropathogenic E. coli (EPEC) belongs to a group of bacterial pathogens that induce epithelial cell actin rearrangements resulting in pedestal formation beneath adherent bacteria. This requires the secretion of specific virulence *proteins"** needed for signal transduction and intimate adherence. EPEC interaction induces tyrosine phosphorylation of a *protein"** in the host membrane, *Hp90"**, which is the receptor for the EPEC outer membrane *protein"**, intimin. *Hp90"**-intimin interaction is essential for intimate attachment and pedestal formation. Here, we demonstrate that *Hp90"** is actually a bacterial *protein"** (*Tir"**). Thus, this bacterial pathogen inserts its own receptor into mammalian cell surfaces, to which it then adheres to trigger additional host signaling events and actin nucleation. It is

also tyrosine-phosphorylated upon transfer into the host cell.

22/3,AB/15 (Item 8 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08760754 GENUINE ARTICLE#: XT420 NUMBER OF REFERENCES: 40
TITLE: Characterization of two virulence proteins secreted by rabbit enteropathogenic Escherichia coli, EspA and EspB, whose maximal expression is sensitive to host body temperature
AUTHOR(S): Abe P; *Kenny B"**; *Stein M"**; Finlay BB (REPRINT)
CORPORATE SOURCE: UNIV BRITISH COLUMBIA,BIOTECHNOL LAB, ROOM 237, WESBROOK BLDG, 6174 UNIV BLVD/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV BRITISH COLUMBIA,BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/; KITASATO INST,DEPT BACTERIOL, MINATO KU/TOKYO 108//JAPAN/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1997, V65, N9 (SEP), P3547-3555
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Enteropathogenic Escherichia coli (EPEC) and rabbit EPEC (RDEC-1) cause unique histopathological features on intestinal mucosa, including attaching/effacing (A/E) lesions. Due to the human specificity of EPEC, RDEC-1 has been used as an animal model to study EPEC pathogenesis. At least two of the previously identified FPEC-secreted proteins, EspA and EspB, are required for triggering host epithelial signal transduction pathways, intimate adherence, and A/E lesions. However, the functions of these secreted proteins and their roles in pathogenesis have not been characterized. To investigate the function of EspA and EspB in RDEC-1, the espA and espB genes were cloned and their sequences were compared to that of EPEC O127, The EspA proteins showed high similarity (88.5% identity), while EspB was heterogeneous in internal regions (69.8% identity). However, RDEC-1 EspB was identical to that of enterohemorrhagic E. coli serotype O26. Mutations in RDEC-1 espA and espB revealed that the corresponding RDEC-1 gene products are essential for triggering of host signal transduction pathways and invasion into HeLa cells. Complementation with plasmids containing FPEC espA or/and espB genes into RDEC-1 mutant strains demonstrated that they were functionally interchangeable, although the FPEC proteins mediated higher levels of invasion. Furthermore, maximal expression of RDEC-1 and EPEC-secreted proteins occurred at their respective host body temperatures, which may contribute to the lack of EPEC infectivity in rabbits.

22/3,AB/16 (Item 9 from file: 440)

07901289 GENUINE ARTICLE#: VR931 NUMBER OF REFERENCES: 56
TITLE: Characterization of EspC, a 110-kilodalton protein secreted by enteropathogenic Escherichia coli which is homologous to members of the immunoglobulinA protease-like family of secreted proteins
AUTHOR(S): *Stein M"**; *Kenny B"**; *Stein MA"**; Finlay BB
CORPORATE SOURCE: UNIV BRITISH COLUMBIA,DEPT BIOCHEM & MOL BIOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV BRITISH COLUMBIA,DEPT BIOCHEM & MOL BIOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/; UNIV BRITISH COLUMBIA,DEPT MICROBIOL & IMMUNOL/VANCOUVER/BC V6T 1Z3/CANADA/
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF BACTERIOLOGY, 1996, V178, N22 (NOV), P6546-6554
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0021-9193
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Enteropathogenic Escherichia coli (EPEC) secretes at least five proteins, Two of these proteins, EspA and EspB (previously called EaeB), activate signal transduction pathways in host epithelial cells. While the role of the other three proteins (39, 40, and 110 kDa) remains undetermined, secretion of all five proteins is under the control of pcrA, a known positive regulator of several EPEC, virulence factors, On the basis of amino-terminal protein sequence data, se cloned and sequenced the gene which encodes the 110-kDasecreted protein and examined its possible role in EPEC signaling and interaction with epithelial cells, In accordance with the terminology used for cspA, and espB, H-e called this gene espC, for EPEC-secreted protein C, We found significant homology between the predicted EspC protein sequence and a family of immunoglobulin A (IgA) protease-like proteins which are widespread among pathogenic bacteria, Members of this protein family are found in avian pathogenic Escherichia coli (Tsh), Haemophilus influenzae (Hap), and Shigella flexneri (SepA). Although these proteins and EspC do not encode IgA protease activity, they have considerable homology with IgA protease fromNeisseria gonorrhoeae and H. influenzae and appear to use a export system for secretion, We found that genes homologous to espC also exist in other pathogenic bacteria which cause attaching and effacing lesions, including Hafnia alvei biotype 19982, Citrobacter freundii biotype 4280, and rabbit diarrheagenic E. coil (RDEC-1). Although these strains secrete various proteins similar in molecular size to the proteins secreted by EPEC, se did not detect secretion of a 110-kDa protein by these strains, To examine the possible role of EspC in EPEC interactions with epithelial cells, we constructed a deletion mutant in espC by allelic exchange and characterized the mutant by standard tissue culture assays, We found that EspC is hot necessary for mediating EPEC-induced signal transduction in HeLa epithelial cells and does not play a role in

adherence or invasion of tissue culture cells.

22/3,AB/17 (Item 10 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

05272639 GENUINE ARTICLE#: MY484 NUMBER OF REFERENCES: 28
TITLE: A DIARRHEAL PATHOGEN, ENTEROPATHOGENIC ESCHERICHIA COLI (EPEC), TRIGGERS A FLUX OF INOSITOL PHOSPHATES IN INFECTED EPITHELIAL CELLS
AUTHOR(S): FOUBISTER V; ROSENSHINE I; *FINLAY BB (Reprint)"**
CORPORATE SOURCE: UNIV BRITISH COLUMBIA,BIOTECHNOL LAB,ROOM 237,WESTBROOK BLDG,6174 UNIV BLVD/VANCOUVER V6T 1Z3/BC/CANADA/ (Reprint); UNIV BRITISH COLUMBIA,BIOTECHNOL LAB/VANCOUVER V6T 1Z3/BC/CANADA/; UNIV BRITISH COLUMBIA,DEPT BIOCHEM/VANCOUVER V6T 1Z3/BC/CANADA/; UNIV BRITISH COLUMBIA,DEPT MICROBIOL/VANCOUVER V6T 1Z3/BC/CANADA/
PUBLICATION: JOURNAL OF EXPERIMENTAL MEDICINE, 1994, V179, N3 (MAR 1), P 993-998
ISSN: 0022-1007
LANGUAGE: ENGLISH DOCUMENT TYPE: NOTE
ABSTRACT: Enteropathogenic Escherichia coli (EPEC) is a bacterial pathogen that causes diarrhea in infants by adhering to intestinal epithelial cells. EPEC induces host cell *protein"** phosphorylation and increases intracellular calcium levels that may function to initiate cytoskeletal rearrangement. We found that EPEC triggers the release of inositol phosphates (IPs) after adherence of bacteria to cultured epithelial cells. We also demonstrated that the EPEC-induced flux of IPs precedes actin rearrangement and bacterial invasion. EPEC mutants and tyrosine *protein"** kinase inhibitors were used to establish that formation of IPs is dependent on tyrosine phosphorylation of a *90"**-*kD"** HeLa *protein"**. Collectively these results suggest that EPEC-induced tyrosine phosphorylation of a host cell substrate(s) leads to release of IPs, which may then trigger cytoskeletal rearrangement.

22/3,AB/18 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01088531
METHODS FOR ASSAYING TYPE III SECRETION INHIBITORS
PROCEDES D'ANALYSE D'INHIBITEURS DE SECRETION DE TYPE III
PATENT ASSIGNEE:
UNIVERSITY OF BRITISH COLUMBIA, (917321), Room 331, IRC Building, 2194 Health Sciences Mall, Vancouver, British Columbia, V6T 1Z3, (CA), (Applicant designated States: all)
INVENTOR:
FINLAY, Brett, B., Biotechnology Lab. 237-6174 University Boulevard,

Vancouver, British Columbia V6T 1Z3, (CA)
*KENNY, Brendan"**, First floor flat 59 Manor Park Redland, Bristol BS6 7HW, (GB)
*STEIN, Marcus"**, Via Fiorentina, II, I-53100 Siena, (IT
PATENT (CC, No, Kind, Date): WO 9945136 990910
APPLICATION (CC, No, Date): EP 99937945 990305; WO 99CA183 990305
PRIORITY (CC, No, Date): US 76980 P 980305
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12Q-001/02; C12Q-001/32; C12Q-001/34; C12Q-001/42; C12Q-001/48; C12Q-001/66; G01N-033/68
LANGUAGE (Publication,Procedural,Application): English; English; English

22/3,AB/19 (Item 2 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01051195
HP90: HOST MEMBRANE RECEPTOR FOR PATHOGENIC BACTERIA, ENCODED BY THE BACTERIAL TIR GENE
HP90:WIRTSREZEPTOR FuR PATHOGENE BAKTERIEN
HP90: RECEPTEUR HOTE A MEMBRANE POUR BACTERIES PATHOGENES CODEES PAR LE GENE BACTERIEN TIR
PATENT ASSIGNEE:
THE UNIVERSITY OF BRITISH COLUMBIA, (917327), 222 Health Science Mall, I.R.C. Building, Vancouver, British Columbia V6T 1Z3, (CA), (Applicant designated States: all)
INVENTOR:
*FINLAY, B., Brett Biotechnology Laboratory"**, 237-6174 University Boulevard, Vancouver, British Columbia V6T 1Z3, (CA)
*KENNY, Brendan Biotechnology Laboratory"**, 237-6174 University Boulevard, Vancouver, British Columbia V6T 1Z3, (CA)
*DEVINNEY, Rebekah Biotechnology Laboratory"**, 237-6174 University Boulevard, Vancouver, British Columbia V6T 1Z3, (CA)
*STEIN, Marcus IRIS CHIRON S.p.A."**, Via Siorentina 1, 53100 Sienna, (IT
LEGAL REPRESENTATIVE:
VOSSIUS & PARTNER (100314), Siebertstrasse 4, 81675 Munchen, (DE)
PATENT (CC, No, Kind, Date): EP 1029054 A1 000823 (Basic)
WO 9924576 990520
APPLICATION (CC, No, Date): EP 98954076 981110; WO 98CA1042 981110
PRIORITY (CC, No, Date): US 65130 971112
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/31; C07K-014/24; C07K-016/12; G01N-033/53; A61K-038/16; C12Q-001/68; C12N-015/62
NOTE:

No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English

22/3,AB/20 (Item 3 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01003446
PATHOGENIC ESCHERICHIA COLI ASSOCIATED PROTEIN
PATHOGENESE-PROTEIN ESPA VON ESCHERICHIA COLI
PROTEINE ASSOCIEE A UN ESCHERICHIA COLI PATHOGENE
PATENT ASSIGNEE:
THE UNIVERSITY OF BRITISH COLUMBIA, (917327), 222 Health Science Mall, I.R.C. Building, Vancouver, British Columbia V6T 1Z3, (CA), (applicant designated states:
AT;BE;CH;DE;DK;ES;FI;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)
INVENTOR:
FINLAY, B. Brett, Biotechnology Laboratory, 237-6174 University Boulevard , Vancouver, British Columbia V6T 1Z3, (CA)
*STEIN, Markus, Biotechnology Laboratory"**, 237-6174 University Boulevard, Vancouver, British Columbia V6T 1Z3, (CA)
*KENNY, Brendan, Biotechnology Laboratory"**, 237-6174 University Boulevard, Vancouver, British Columbia V6T 1Z3, (CA
LEGAL REPRESENTATIVE:
VOSSIUS & PARTNER (100311), Postfach 86 07 67, 81634 Munchen, (DE)
PATENT (CC, No, Kind, Date): EP 904288 A2 990331 (Basic)
WO 9740063 971030
APPLICATION (CC, No, Date): EP 97917185 970423; WO 97CA265
PRIORITY (CC, No, Date): US 15999 P 960423
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C07K-014/00
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English

22/3,AB/21 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0243573 DBA Accession No.: 1999-14338 PATENT
Identifying antibacterial agents that inhibit Gram-negative type-III secretion system, for treating infections -by screening for inhibition of virulence factors secreted by this system - e.g. plasmid pMS21-mediated EspB gene, herpes simplex virus tag gene transfer and expression in Escherichia coli

AUTHOR: Finlay B B; *Kenny B"**; *Stein M"**
CORPORATE SOURCE: Vancouver, British Columbia, Canada.
PATENT ASSIGNEE: Univ.British-Columbia 1999
PATENT NUMBER: WO 9945136 PATENT DATE: 19990910 WPI ACCESSION NO.: 1999-540860 (1945)
PRIORITY APPLIC. NO.: US 76980 APPLIC. DATE: 19980305
NATIONAL APPLIC. NO.: WO 99CA183 APPLIC. DATE: 19990305
LANGUAGE: English
ABSTRACT: Identification of antibacterial agents is new and involves treating bacteria that contain a polynucleotide which encodes a protein secreted by the type-III secretion system (3SS) with a test compound and detecting secretion of the protein. A reduction of secretion, relative to that in bacteria not treated with the test compound, indicates an inhibitor of 3SS. Also claimed is a kit containing in separate containers, the bacteria and a system for detecting secretion of the protein. The antibacterial agents can be used to treat infections in humans other animals and plants, e.g. where caused by enteropathogenic or enterohemorrhagic Escherichia coli, Yershi sp., Shigella sp., Pseudomonas aeruginosa, Pseudomonas syringae, Xanthomonas campestris or many others, for analyzing the functional mechanisms of 3SS and for development of more powerful or specific inhibitors. In an example, plasmid pMS21 containing a sequence encoding the N-terminal part of protein EspB and a sequence encoding a herpes simplex virus tag against which commercial antibiotics are available was used to transform 2 enteropathogenic strains of Escherichia coli to test for inhibitors. (51pp)

22/3,AB/22 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0219264 DBA Accession No.: 98-00861 PATENT
EspA from enteropathogenic or enterohemorrhagic Escherichia coli - vector expression in host cell for recombinant protein production for use as a recombinant vaccine
AUTHOR: Finlay B B; *Stein M"**; *Kenny B"**
CORPORATE SOURCE: Vancouver, British Columbia, Canada.
PATENT ASSIGNEE: Univ.British-Columbia 1997
PATENT NUMBER: WO 9740063 PATENT DATE: 971030 WPI ACCESSION NO.: 97-535772 (9749)
PRIORITY APPLIC. NO.: US 15999 APPLIC. DATE: 960423
NATIONAL APPLIC. NO.: WO 97CA265 APPLIC. DATE: 970423
LANGUAGE: English
ABSTRACT: A new secreted EspA protein from Escherichia coli with a mol.wt. of 25,000 by SDS-PAGE is encoded by DNA (protein and DNA sequence specified) which can be contained on a vector and used to transform a host cell for production of the recombinant protein. Also claimed is an

polyclonal or monoclonal antibody which binds to the EspA protein and which can be used to detect EspA in a tissue or biological fluid sample. The presence of EspA indicates infection by enteropathic E. coli. The protein may be used to immunize a host against disease caused by EspA-producing E. coli, or ameliorating such a disease. A DNA probe that hybridizes to the espA nucleic acid molecule can be used to detect espA in a sample. Also claimed is a method of identifying a compound which inhibits bacterial type-II secretion systems, a method for producing a nonpathogenic organism, preferably E. coli, and a method of producing a fusion protein containing EspA and a target protein. (62pp)

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